University of Alberta

Systematics and Development within the Sea Spiders (Arthropoda: Pycnogonida) from the Northeastern Pacific.

by

Jayson Matthew Gillespie

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

Pycnogonida, or sea spiders (Arthropoda) may represent the earliest branch of arthropod phylogeny, but to date little is known about their biology and relationships with other arthropods. This thesis addresses three aspects of pycnogonid biology and systematics where current knowledge is lacking: a new species description, postembryonic development, and family-level molecular phylogenetics. The new species, *Tanystylum bealensis* is the most common pycnogonid found along the coast of British Columbia. Development of this species consists of nine instars from egg to adult, with defining characteristics being addition of walking legs and modification of head appendages. Molecular phylogenetic analysis of pycnogonid families using 18S and 28S rDNA demonstrated that traditional classifications were poorly supported, but was unable to provide much improved resolution. By increasing our knowledge of pycnogonid systematics and development, we will begin to understand this unique group and ultimately gain insight into arthropod evolution.

"The only justifiable conclusion, and one that cannot offend any national sensibilities, is that the total pycnogonid population of the overall world ocean has increased threefold since 1948. At this rate of increase (i.e., about 60% per annum) it is estimated that the pycnogonids may support a major fishery sometime in the next millennium."

-- Joel Hedgpeth. 1954. Systematic Zoology 8(4): 147 --

"But the beard seemed to melt away as she touched it, and she found herself sitting quietly under a tree -- while the Gnat (for that was the insect she had been talking to) was balancing itself on a twig just over her head, and fanning her with its wings. It certainly was a very large Gnat: `about the size of a chicken,' Alice thought. Still, she couldn't feel nervous with it, after they had been talking together so long.

` -- then you don't like all insects?' the Gnat went on, as quietly as if nothing had happened.

`I like them when they can talk,' Alice said. `None of them ever talk, where I come from.'

`What sort of insects do you rejoice in, where you come from?' the Gnat inquired.

`I don't rejoice in insects at all,' Alice explained, `because I'm rather afraid of them -- at least the large kinds. But I can tell you the names of some of them.'

`Of course they answer to their names?' the Gnat remarked carelessly.

`I never knew them do it.'

`What's the use of their having names the Gnat said, `if they won't answer to them?'

`No use to them,' said Alice; `but it's useful to the people who name them, I suppose. If not, why do things have names at all?' "

-- Alice and the Gnat [Dodgson, C. L. (AKA Lewis Carrol). 1871. Through the Looking Glass and What Alice Found There - Chapter 3: Looking-Glass Insects.] --

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CHAPTER ONE

General Introduction

1.1 Overview

The Pycnogonida (Gr. *pyknos* = crowded + *gony* = knee), or sea spiders (Phylum Arthropoda) are a small unique group of exclusively marine spider-like organisms. Historically, they have also been referred to as the Pantopoda or Podosomata. Rarely found in high densities, often cryptic, and of no known economic importance, pycnogonids have received little more than trivial coverage in the scientific literature and general zoology texts (Hedgpeth 1949; Arnaud and Bamber 1987; Ruppert and Barnes, 1994; Brusca and Brusca 2002; Arango 2003). They are found in every ocean around the world at depths ranging from intertidal to abyssal (>7000m), but many species are found in littoral or shallow-waters (Hedgpeth 1982). Although quite common in most habitats, pycnogonids are often overlooked due to their slow movements, generally small size, and good camouflage (Berry 1980; Arnaud and Bamber 1987; Bain 1992). Most species of pycnogonids are small, with a leg span of less than one centimeter, but some deep-sea species have leg spans of up to 70 cm (Arnaud and Bamber 1987; Lovely 1999; Arango 2002). While most are epibenthic, some are interstitial, bathypelagic, commensal and even parasitic (Child and Harbison 1986; Arnaud and Bamber 1987; Russell and Hedgpeth 1990). Pycnogonids typically feed on sessile or slow moving prey such as sponges, cnidarians, or bryozoans (Arnaud and Bamber 1987). They have also been reported to feed on lamellibranch bivalves, gastropods, echinoderms, polychaetes as well as algae and organic detritus. However, at least one species is capable of capturing rapidly moving brine shrimp (Bain 1991). Several aspects of their biology make them distinct from other arthropods, including: 1) the lack of a respiratory or excretory system; 2) possession of the distinct, non-homologous proboscis and ovigerous legs; 3) presence of multiple gonopores; 4) chemical compounds not present in any other animal group; 5) and they are the only invertebrate group to possess exclusive paternal care (Ridley 1978; Hedgpeth 1982; Arnaud and Bamber 1987; Tomaschko 1997; Arango 2003).

Many aspects of pycnogonid biology remain poorly known, and as a result, we may not fully appreciate the significance of pycnogonids in arthropod evolution. The systematics of pycnogonids is so poorly resolved that taxonomic descriptions of new species remain the most common form of publication on sea spiders (Arango 2003). Phylogenetic studies of relationships within the pycnogonids have been largely based on morphology, yet because too few characters are available, little has been resolved (Bain 1992; Lovely 1999; Arango 2002). Developmental patterns have enhanced our understanding of phylogenetic relations and character evolution, yet we know very little about pycnogonid development (see Bain 2003). Molecular data have also become a useful tool for addressing phylogenetic relations, yet molecular work on the pycnogonids has just begun (Arango 2003). The homology of head appendages, one of the key characteristics used to understand relationships among arthropod subphyla, is still unclear in the pycnogonids. Their head appendages have distinct names compared to other arthropod subphyla and also to other chelicerates like arachnids (i.e., chelifores vs. chelicerae; palps vs. pedipalps) (Bain 1992). This thesis addresses three aspects of pycnogonid biology where our current knowledge is lacking: taxonomy, development, and molecular phylogenetics.

1.2 Pycnogonid Morphology

For most people, the first impression of a pycnogonid is that it is all legs, but has no body (Cadien 1997). So decentralized is their appearance, that the Reverend T.R.R. Stebbing used the term "nobodies" as a popular name for the group (Stebbing 1902; Cadien 1997). The basic pycnogonid body is considerably reduced, and comprised of five segments (Fig. 1-1). The first four segments comprise the prosoma or cephalothorax, each bearing a pair of walking legs, while the last segment is the opisthosoma or abdomen. However, in a small number of species (polymerous forms), the prosoma consists of five or six body segments (Hedgpeth 1947; Bain 1992).

The first segment, the cephalon, bears the proboscis, the ocular tubercle and three pairs of appendages: the chelifores, the palps, and the ovigerous legs or ovigers (Arnaud and Bamber 1987). The proboscis is probably the most prominent external feature of the group, but varies considerably in shape and size among the different families, genera and species. It is the feeding organ, and its form reflects prey choice (Cadien 1997). The ocular tubercle, on which the eyes are situated, varies in shape and position on the cephalon. It bears two pairs of eyes, but in some abyssal or psammophilic species the eyes are lacking or the entire ocular tubercle is absent (Arnaud and Bamber 1987; Cadien 1997). The chelifores are comprised of a basal scape with one or two segments and a chela with one moveable finger articulating on the palm of the fixed finger (Arnaud and Bamber 1987; Cadien 1997). This appendage is present in all larvae, but shows various degrees of reduction in adults among the different families. The palps are used for sensory, feeding and cleaning functions (Arnaud and Bamber 1987). They range from being completely absent to being twenty segmented. The ovigers are specialized cleaning appendages, and are used by the males to carry the eggs and sometimes the larvae. They are found in males of almost species, but vary with families as to their presence in females. In families where females have ovigers, they are typically smaller in size in relation to the male's ovigers (King 1973). The presence or absence, and the number and configuration of segments in the chelifores, palps and ovigers are important diagnostic characteristics in pycnogonid taxonomy (Arnaud and Bamber 1987).

Each segment of the cephalothorax has two prominent lateral processes on which the walking legs articulate. Most species have four pairs of walking legs, though polymerous species have five or six pairs (Hedgpeth 1947; Bain 1992). The walking legs are eight segmented, with three coxae, a femur, two tibiae, a tarsus and a propodus (King 1973; Arnaud and Bamber 1987). The propodus typically bears two sets of claws and two sets of spines, but this depends on family, genus and species. The claws include the single main and paired auxiliary claws, and the spines included the sole and heel spines. The second coxae of the walking legs bear the gonopores (sexual pores), usually opening on the ventral surface. Females typically have gonopores present on all four pairs of walking legs, while males tend to have gonopores only on the third and fourth walking legs, and occasionally on the second walking legs. The femur of males contains specialized glands called the cement glands. These are secretory glands that produce cement that glue the fertilized eggs to the male's ovigers (King 1973; Arnaud and

Bamber 1987). The cement glands open via pores on the surface of the femur, which are variable in number and morphology, and are taxonomically diagnostic.

The opisthosoma (abdomen) is highly reduced compared to all other arthropods and may be articulating or fixed. It typically bears spines, but has no abdominal appendages (Cadien 1997). It bears the anal opening.

Internal anatomy of the pycnogonids is relatively simple. They possess neither a respiratory nor an excretory system (Hedgpeth 1949; King 1973; Arnaud and Bamber 1987; Cadien 1997). This is likely due to their large surface area to volume ratio as a result of their peculiar morphology. Gas exchange and excretion are assumed to occur through the integument or gut wall (King 1973; Hedgpeth 1982; Arnaud and Bamber 1987). The digestive system runs from the mouth at the tip of the proboscis, into the esophagus (foregut), through the prosoma segments (midgut), into the abdomen (hindgut) and out through the anus (Arnaud and Bamber 1987; Berry 1980; Cadien 1997). However, due to the limited space within the prosoma, the midgut extends into the appendages and legs. The circulatory system is open and centered on a middorsal heart that acts more as a channel than a pump (Arnaud and Bamber 1987). The majority of circulation is derived from gut peristalsis and leg movement, particularly in species with long legs (Helfer and Schlottke 1935; King 1973). The nervous system is that of a typical arthropod, with a supraesophageal ganglion (brain) connected by circumesophageal commissures to a chain of paired ventral ganglia (King 1973; Berry 1980). The ventral ganglia correspond to the ovigers and each pair of walking legs (Arnaud and Bamber 1987). The supraesophageal ganglion consists of a protocerebrum and a tritocerebrum and lacks a deutocerebrum, a characteristic in the arthropods shared only with the chelicerates (Brusca and Brusca 2002). The reproductive system is simple and similar between males and females. In both sexes, there is one U-shaped gonad situated above the heart with diverticulata extending into the walking legs as far as the femur (Berry 1980; Arnaud and Bamber 1987; Cadien 1997). Ova are stored in the femurs of all legs until their release just prior to fertilization via the gonopores.

1.3 Reproduction in Pycnogonids

Reproduction in pycnogonids is poorly understood and is one area currently being researched. Pycnogonid reproduction is unique from all other invertebrates, as they are the only group known to possess exclusive paternal care (Ridley 1978). Mating has only been observed in a few species, but even this limited view indicates a great deal of variation within the group, and is likely dictated by the positions of the gonopores (Cadien 1997). In *Anoplodactylus lentus*, where the gonopores of both the male and female are on the ventral surface, pairing is ventrum to ventrum (Cole 1901; Cadien 1997). In *Pycnogonum littorale*, the male gonopores open on the ventral surface of the leg, while the gonopores of the female open on the dorsal surface; thus the pairing is ventrum to dorsum (Jarvis and King 1972; King and El-Hawawi 1978; Cadien 1997).

As the female extrudes eggs, they are assumed to be fertilized externally by the male as no copulatory organs exist and fertilization has ever been observed (Berry 1980). The male then gathers the eggs into a ball and attaches them to his ovigers using cement from femoral cement glands (Arnaud and Bamber 1987). The male carries the eggs until hatching and in some groups until several instars have passed. To demonstrate our general lack of knowledge in regards to pycnogonid reproduction, we need only consider the Colossendeidae, one of the nine families of pycnogonids. Although 89 species in six genera are known from this family, nothing is known about their reproduction; no males have ever been found carrying eggs or larvae (Arnaud and Bamber 1987).

Embryonic development follows a typical arthropod pattern and is discussed in detail by King (1973) and Arnaud and Bamber (1987). Bain (2003) has recently summarized the postembryonic development within the pycnogonids, and illustrates that complete postembryonic development has been described for only a handful of species, while descriptions of isolated larval stages are more commonly reported in the literature.

1.4 Taxonomy and Systematics of Pycnogonids

The phylogenetic position of the Pycnogonida has always been an enigma. Since their discovery, very little research has been done on this unusual group of organisms, thus their classification remains problematic (Arnaud and Bamber 1987). For example, Linnaeus originally placed pycnogonids in the genus *Phalangium*, along with the terrestrial spiders. Eventually, they were removed from this genus and placed in a taxon by themselves. Currently pycnogonids are placed within the phylum Arthropoda, although their systematic positioning is still a matter of great debate. The pycnogonids have been associated at one time or another with virtually every major group of arthropods (King 1973), as well as with the onychophorans (Tiegs and Manton 1958) and the polychaete annelids (Henry 1953). Arnaud and Bamber (1987), recognizing the similarities and differences between the pycnogonids and chelicerates, suggested that the best available alternative was the consideration of the Pycnogonida as a unique and distinct subphylum of the Arthropoda. Kozloff (1990) even suggested it best to consider the pycnogonids as an anomalous group whose status is currently uncertain. Most recent authors place the pycnogonids within the subphylum Chelicerata as the class Pycnogonida, alongside the horseshoe crabs (Class Merostomata) and the arachnids (Class Arachnida) (Giribet and Ribera 1998; Wheeler and Hayashi 1998; Regier and Shultz 2001; Brusca and Brusca 2002). Regardless of their position, a better understanding of this group is needed to help resolve the evolutionary history of the arthropods (Regier and Shultz 1997; Fortey and Thomas 1998).

Current pycnogonid classifications are based largely on the work of Joel Hedgpeth. Hedgpeth (1947) divided the pycnogonids into eight families on the basis of the presence or absence and number of segments of the chelifores, palps and ovigers. With a few modifications by Stock (1954), Hedgpeth and McCain (1971), and Fry (1978), this classification remains in use today. However, nine families are now recognized: Ammotheidae, Austrodecidae, Callipallenidae, Colossendeidae, Endeidae, Nymphonidae, Phoxichilidiidae, Pycnogonidae, Rhynchothoracidae (Table 1-1). Hedgpeth (1954) proposed ordinal ranks to divide the pycnogonids into two groups, the order Palaeopantopoda to cover all fossil pycnogonids, and the order Pantopoda for all living species. However, these ordinal ranks have not been generally accepted. The pycnogonid fossil record is represented by only three known species, all from the Devonian (see Arnaud and Bamber 1987). A fourth possible species, a larva from the Cambrian is currently being described (Walossek and Dunlop, in press). Although generally acknowledged to not represent the true evolutionary relationships, an acceptable alternative classification has yet to be proposed, leaving the traditional classification to be used for a matter of convenience and stability.

1.5 This Study

Although the topics are somewhat eclectic, the chapters in this thesis all address important aspects of pycnogonid biology, including alpha taxonomy, development, and phylogenetic relations of major groups. In chapter 2, I describe a new species of pycnogonid, *Tanystylum bealensis*, from Barkley Sound, Vancouver Island, British Columbia, Canada. In chapter 3, I continued my work on the species, *T. bealensis*, by describing its complete postembryonic development. This is the first complete postembryonic development of a pycnogonid from a field collection. In chapter 4, I examine the phylogenetic relationships of 36 species representing eight families using two nuclear genes (18S and 28S ribosomal DNA). This is the second molecular phylogeny of the pycnogonids and includes all available sequences and taxa from Genbank and previous work, making it the largest dataset analyzed. In chapter 5, I provide a general summary of this thesis and its implications for future research on pycnogonids. Finally, as an appendix, I provide a checklist to the pycnogonids available at the Royal British Columbia Museum in Victoria, B.C., Canada, as well as my notes on pycnogonid taxonomy and diversity to the families and genera.

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Table 1-1. Diagnostic characteristics for the nine pycnogonid families.

Family	Chelifores	Palps		Ovigers	
	Present/Absent	Present/Absent	# Segments	Present in Females	# Segments
Ammotheidae	Both	Present	4-10	Present	9-10
Austrodecidae	Absent	Present	5-8	Present	4-10
Callipallenidae	Present	Present (Males only)	0,1 or 4	Present	9-10
Colossendeidae	Absent	Present	10	Present	10
Endeidae	Absent	Absent	--	Absent	7
Nymphonidae	Present	Present	4-5	Present	10
Phoxichilidiidae	Present	Absent	--	Absent	5-6
Pycnogonidae	Absent	Absent	--	Absent	9
Rhynchothoracidae	Absent	Present	4	Present	8

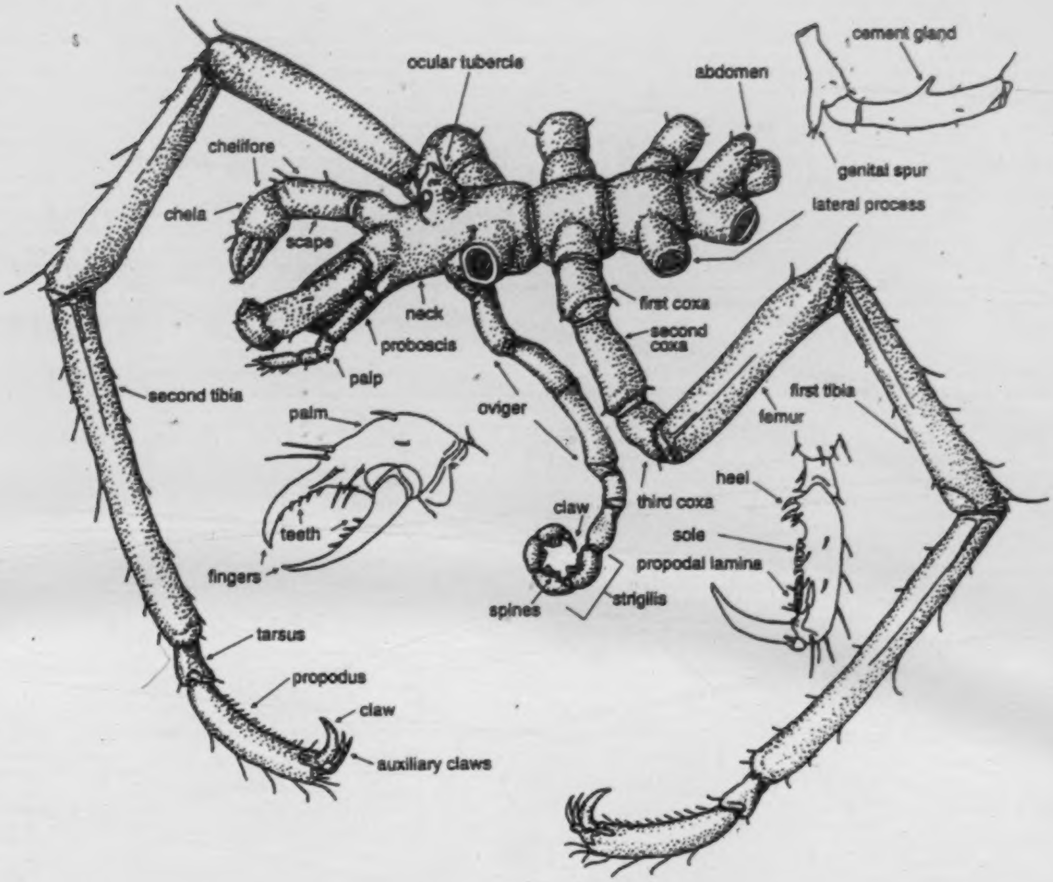
ocular tubercle
abdomen
cement gland
chelifore
genital spur
chela
lateral process
scape
neck
first coxa
second coxa
proboscis
palp
second tibia
palm
oviger
first tibia
femur
heel
third coxa
claw
teeth
sole
propodal lamina
fingers
strigilis
spines
tarsus
propodus
claw
auxiliary claws

Figure 1-1. Diagram of the general pycnogonid morphology, showing structures described in the text. Taken from Child (1998).

CHAPTER TWO*

Tanystylum bealensis, a new species of sea-spider (Arthropoda: Pycnogonida: Ammotheidae) with a key to *Tanystylum* species from the Pacific Coast of North America.

2.1 Introduction

The pycnogonids of the west coast of North America remain poorly known and in need of a thorough reexamination (Child 1990). Much of the literature dealing with the pycnogonid fauna of this region is outdated and many of the type descriptions are inadequate. This problem was recently highlighted by the discovery of a *Tanystylum* species in Barkley Sound, British Columbia, Canada. The only *Tanystylum* species previously described from this region was *T. occidentalis* (Cole, 1904) according to Austin (1983).

Initially, when compared to the type description for *T. occidentalis*, the newly collected *Tanystylum* specimens appeared to match this species. However, subsequent detailed examination of the *T. occidentalis* type material revealed significant differences between the two. This paper describes the new species, *Tanystylum bealensis*, and provides a detailed comparison with its closest relative, *Tanystylum occidentalis*.

2.2 Materials and Methods

2.2.1 Material Examined

Specimens were obtained from both museum collections (16 females, 17 males) and the field (213 females, 244 males). Museum specimens were examined from the National Museum of Natural History, Smithsonian Institution (Washington, D.C. USA, 20560-0163; USNM), and the Royal British Columbia Museum (675 Belleville Street, Victoria, British Columbia, Canada, V8W 9W2; RBCM).

In the field, *Tanystylum bealensis* specimens were consistently found on the hydroid *Plumularia setacea* (Ellis, 1755). Both pycnogonids and hydroids were found together at four different localities (Cape Beale, Bordelais Islet, Edward King Island, Cree Island) in Barkley Sound, Vancouver Island, British Columbia, Canada over two years (May-August 1998 and [8]1999). Both pycnogonids and hydroids were preserved in a ten percent seawater-formalin solution. Individual pycnogonids were removed from the hydroid and sorted under a Wild M3B stereo microscope at 40X magnification. Specimens were deposited at the National Museum of Natural History, Smithsonian Institution (USNM), Canadian Museum of Nature (P.O. BOX 3443, Station D, Ottawa, Ontario, Canada, K1P 6P4; CMNC), Royal British Columbia Museum (RBCM) and Bamfield Marine Station (Bamfield, British Columbia, Canada, V0R 1B0; BMSM).

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* This chapter has been co-authored with Dr. Bonnie Bain and has been accepted for publication in the journal 'Species Diversity'.

2.2.2 Specimen Preparation for SEM

Nine adult male and five adult female pycnogonids from Cape Beale, Barkley Sound were ultrasonically cleaned, run either through an ethanol-hexamethyldisilazane (HMDS) dehydration series or through an ethanol dehydration series and critical-point dried, placed onto 1.27 cm diameter aluminum stubs, and coated with 20 nm gold in a SEM Prep 2 sputter coater. SEM observation was done in a Hitachi S450 scanning electron microscope at 10-20kV (U. Calgary) or in a JEOL 6301F field emission scanning electron microscope at 10kV (U. Alberta). All drawings were made from SEM images.

2.2.3 Measurements

The largest male specimen of *Tanystylum bealensis* seen from the type locality (Cape Beale, Barkley Sound, BC; RBCM 002-00144-001) and the holotype of *Tanystylum occidentalis* (Cole, 1904; USNM 1074913) were measured with the aid of a camera lucida attached to a Wild S5 stereo microscope, a graphics tablet and MacMeasure II, Version 2.35 (Rasband and Palmer 1993). Each structure of each specimen was measured twice to give an indication of measurement error. After measuring the body (trunk length, trunk width, abdomen length, proboscis length), the *T. bealensis* specimen was dissected and mounted on a slide. This was done to allow all segments of all appendages to be measured. All measurements taken were linear and include: trunk length (from chelifore insertion to tip of fourth lateral processes); trunk width (across second lateral processes); abdomen length (base to tip, when viewed dorsally); proboscis length (from tip to base of collar, when viewed ventrally); palp length (from proximal end of segment (at midpoint) to distal end of segment (at midpoint), when viewed laterally); oviger length (from proximal end of segment (at midpoint) to distal end of segment (at midpoint), when viewed laterally); and length of individual segments of each walking leg (coxae one, two, and three; femur; tibiae one and two; tarsus; propodus; main claw; auxiliary claw) measured from proximal end of segment (at midpoint) to distal end of segment (at midpoint), when viewed laterally.

Ten individual adult male specimens of *Tanystylum bealensis* (holotype, USNM XXXX; two specimens, USNM XXXX; two specimens, CMNC 2000-0001; one specimen, RBCM 002-00144-001; one specimen, RBCM 973-00159-013; one specimen, RBCM 973-00249-017; two specimens, RBCM 973-00249-002) were measured to provide an indication of intraspecific variation. Since dissection of specimens was required to measure all segments for all appendages, only structures measureable on whole specimens were used for intraspecific variation. The structures chosen were based on ease of measurement and presence in all specimens. These structures (described above) include: trunk length, trunk width, abdomen length, proboscis length, length of palp segment five, length of oviger segments four and five, and length of third right walking leg segments (femur, tibia 1, tibia 2).

2.3 Results

Family **Ammotheidae** Dohrn, 1881
Genus ***Tanystylum*** Miers, 1879
***Tanystylum bealensis*,** new species
(Figs. 2-1 to 2-7)

2.3.1 Diagnosis

Ocular tubercle located in middle of cephalic segment. Proboscis with distinct collar at base; shape slightly conical, tapering with convex sides towards mouth. Chelifores one-segmented, not connected at base. Palps five-segmented (rarely four-segmented), longer than length of proboscis. Abdomen with basal, dorsal mound. Male gonopores present on second pair of walking legs.

2.3.2 Material Examined

Holotype: male (USNM XXXXXX), Canada, British Columbia, Vancouver Island, Barkley Sound, Cape Beale, 48°47'1"N, 125°13'51"W, littoral, 0.0-0.6 m tidal height on wave-exposed rocky shore, 21 August 1998, coll. J.M. Gillespie. Allotype: female (USNM XXXXXX), Canada, British Columbia, Vancouver Island, Barkley Sound, Cape Beale, 48°47'1"N, 125°13'51"W, littoral, 0.0-0.6 m tidal height on wave-exposed rocky shore, 21 August 1998, coll. J.M. Gillespie. Paratypes: two females, two males, two males with eggs (USNM XXXXXX); 16 juveniles (USNM XXXXXX); two females, two males, two males with eggs (CMNC 2000-0001); 16 juveniles (CMNC 2000-0002); two females, two males, two males with eggs, 15 juveniles (RBCM 000-00036-001); two females, two males, two males with eggs (BMS #IL2281); 15 juveniles (BMS # IL2282); one male, slide mount of appendages only (RBCM 002-00144-001), Canada, British Columbia, Vancouver Island, Barkley Sound, Cape Beale, 48°47'1"N, 125°13'51"W, littoral, 0.0-0.6 m tidal height on wave-exposed rocky shore, 21 August 1998, coll. J.M. Gillespie. Two females, two males (RBCM 002-00003-001), Barkley Sound, Bordelais Islets, 48°49'6"N, 125°14'48"W, littoral, 0.0 - 0.6 m tidal height, 15 June 1999, coll. J. M. Gillespie. One male with eggs, three juveniles (RBCM 002-00002-001), Barkley Sound, Cree Island, 48°51'3"N, 125°20'45"W, littoral, 0.0 - 0.6 m tidal height, 16 July 1999, coll. J. M. Gillespie. Two females, two males (RBCM 002-00001-001), Edward King Island, 48°50'24"N, 125°13'48"W, subtidal, 10m, 7 August 1999, coll. J. M. Gillespie. Non-types: One female (USNM 69493); subtidal (39.1 m), Bonita Point Light, San Francisco Bay, California, USA, 37°49'N, 122°31.5'W; 4 November 1912; coll. L. Giltay. One female (USNM 80973); littoral, rocky tidal flats of Pysht, Olympic Peninsula, Washington, USA, 48°12'N, 124°06'W; 26 July 1930; coll. E. Ricketts and W.A. Hilton. Two females (USNM 80976); 15 miles south of Pacific Grove, California, USA, 36°27'N, 121°56'W; 29 May 1930; coll. E. Ricketts and W.A. Hilton. One male (RBCM 973-00159-013); littoral, S.E. Howell Island, Barkley Sound, Vancouver Island, British Columbia, Canada, 48°51'30"N, 125°20'30"W; 1 July 1973;

coll. P. Lambert. Seven females, two males, six males with eggs, six juveniles (RBCM 973-00249-017); five females, five males, 4 males with eggs, 18 juveniles (RBCM 973-00249-002); subtidal (12.1 m), Race Rocks, Vancouver Island, British Columbia, Canada, 48°18'N, 123°32'W; 6 September 1973; coll. P. Lambert and B. Cooke. One juvenile (RBCM 976-01081-026); littoral, Moresby Island, Tasu Sound, Queen Charlotte Islands, British Columbia, Canada, 52°45'00"N, 132°05'42"W; 21 August 1976; coll. P. Lambert.

2.3.3 Distribution and Habitat

In all collections from Barkley Sound, British Columbia, *Tanystylum bealensis* was found only on exposed shores, including the littoral zone of Cape Beale, Bordelais Islets, and Cree Island, and in the subtidal zone (10 m depth) from Edward King Island, Barkley Sound. In all cases, *T. bealensis* was found associated with the hydroid *Plumularia setacea.* At all littoral locations examined, *P. setacea* was found to be growing on the mussel *Mytilus californianus* Conrad, 1837, while at the subtidal site at Edward King Island, the hydroid was found growing on bedrock. Less exposed shores with *Mytilus californianus* (e.g. Wizard Islets, Ross Islets and Eagle Bay, Barkley Sound) were examined for both *Tanystylum bealensis* and the hydroid *Plumularia setacea*, but neither were found.

Museum and field collections indicate the distribution of *Tanystylum bealensis* is from Pacific Grove, California, USA to the Queen Charlotte Islands, British Columbia, Canada. Habitat information is not available from museum collections except for two from Race Rocks, British Columbia, Canada (RBCM 973-00249-017 & RBCM 973-00249-002) and one from the Queen Charlotte Islands (RBCM 976-01081-026), where the hydroid *Plumularia setacea* was associated with the specimens.

2.3.4 Description

Trunk (Figs. 2-2A,B) circular, unsegmented, smooth; lateral processes closely crowded, each with one to three (typically two or three, last leg usually one) dorsal spines; lines between lateral processes radiating toward common point slightly anterior of abdomen; lateral processes of females only 75% length of males; anterior of cephalic segment without lateral tubercles or spines.

Abdomen (Figs. 2-2A,B) varies in orientation, from i) extending beyond lateral processes to first coxae of fourth leg, half height of ocular tubercle to ii) extending to tips of lateral processes, one and a half times height of ocular tubercle; proximal half of segment as small mound, directed straight back; distal half curved upward; base and tip armed with five to six and six spines respectively.

Ocular tubercle (Figs. 2-2A,B) located in middle of cephalic segment; truncated cone half as tall as wide, capped anteriorly with small truncated tubercle bearing four red eyes.

Proboscis (Figs. 2-2C; 2-3) arises from short collar-like extension of cephalic segment, broad at its base and tapering with convex sides towards mouth, twice as long as broad; directed straight forward or anterio-ventrally; tri-radiate mouth with hair-like projections along inner edges of lips.

Palps (Fig. 2-4A) five-segmented, rarely four-segmented; extend past tip of proboscis; segment two armed with single dorsal spine on distal end; segment three longest, armed on distal end with one lateral and one ventral spine; segment four with five ventral spines at distal end; segment five with two groups of ventral spines, two thicker lateral spines, and several longer spines on anterior end.

Chelifores (Fig. 2-4B) one-segmented, short, bulbous, not joined together at base; each with four dorso-distal spines.

Ovigers ten-segmented in both sexes; female ovigers shorter than male ovigers. *Male ovigers* (Figs. 2-4C,D): segment one short and bulbous; segment two more elongate, up to twice length of segment one, with three to five short ventral spines; segment three approximately same length as segment one, with one short ventral spine; segment four approximately one and half to two times length of segment three; segment five approximately same length or longer than segment two, slightly curved; segment six shorter, approximately half length of segment five, with two long and one short terminal spine and several thicker lateral spines; segments seven, eight, and nine much shorter than preceding segments, curved into modified strigilis; segment seven attached to three-quarters of segment six, lacking apophysis; segments seven and nine with pair of subterminal spines; segment eight with three terminal spines; segment ten small rounded knob with pair of elongate forked terminal spines. *Female ovigers* (Fig. 2-4E): segments one to six similar to male, but each segment is half length of equivalent male segment; segments seven to nine short and round; segments seven and nine each with single mid-segmental spine and segment eight with pair of mid-segmental spines; segment ten very short with pair of short, elaborately forked terminal spines.

Walking legs (Figs. 2-4F,G) lengths for each segment highly variable; coxa one (segment #1) short, three to five spines on dorso-distal surface, small anterior processes on distal lateral edges; coxa two one and half times length of coxa one; coxa three approximately equal in length to coxa one; femur approximately four-fifths length of combined coxal lengths, dorsal surface with one or two mid-segmental spines and with prominent distal projection surmounted by six to ten spines, ventral surface with two pairs of spines, one pair proximal and other pair mid-segmental and located on low mound; tibia one slightly smaller in length than femur, with three or four low dorsal mounds, all but most distal mound crowned with three or four spines, ventral surface with several scattered distal spines; tibia two approximately equal or slightly longer than femur length, with three low dorsal mounds, each with several spines, ventral and lateral surfaces of tibia two each have three shorter spines; tarsus with eight short spines on ventro-distal edge, one to three short spines arising proximally in parallel orientation; propodus with three stout heel spines and typically 13 sole spines, number of sole spines varies from 10 to 13 especially on fourth walking leg; main claw half propodal length; auxiliary claws half length of main claw.

Gonopores (Fig. 2-5) of male located on ventral side of coxa two of walking legs two, three, four. Female gonopores located on ventral side of coxa two on all four pairs of walking legs.

Cement Gland (Fig. 2-6) of males located between distal spines and middle spines on dorsal side of femur of all four pairs of walking legs. Cement glands not present in females.

Spines (Fig. 2-7) of body and appendages hollow tipped with bifurcated sides; includes all spines on both males and females except heel and larger sole spines of propodus and terminal spines of ovigers.

Color opaque white in both live and preserved specimens.

2.3.5 *Measurements*

Two duplicate measures of all structures were made for a single specimen of *T. bealensis* n.sp. and the *T. occidentalis* holotype, and show that for all structures *T. occidentalis* is larger in size than *T. bealensis* (Tables 2-1 and 2-2). Comparison of the holotype of *T. occidentalis* with the intraspecific variation of *T. bealensis* shows that *T. occidentalis* falls outside of the range of intraspecific variation of *T. bealensis* (Table 2-3).

2.3.6 *Etymology*

The name proposed for this species, *bealensis,* refers to the type locality (Cape Beale, Barkley Sound, British Columbia, Canada) where this species was first discovered.

2.3.7 *Remarks*

Of the seven known *Tanystylum* species from the Pacific coast of North America (Canada and United States, including Alaska), *T. bealensis* most closely resembles *T. occidentalis*. Both share the following characteristics: one-segmented chelifores, eye tubercle located in the middle of the cephalic segment, male gonopores present on the second walking leg and the absence of concave sides on the proboscis. These characteristics separate them from the other five remaining Pacific North American *Tanystylum* species: *T. californicum* Hilton, 1939; *T. duospinum* Hilton, 1939; *T. grossifemora* (Hilton, 1942); *T. intermedium* Cole, 1904; *T. nudum* Hilton, 1939. A key to the seven *Tanystylum* species from the North American Pacific (Canada and United States, including Alaska) is given below.

Table 2-4 lists all the characteristics for which *Tanystylum bealensis* is distinct from *T. occidentalis*. The most important of these are: presence of a distinct collar at the base of the proboscis, chelifores not connected at the base, five palp segments (instead of four), palps longer than proboscis, and cement gland located three-quarters along the length of the femur rather than at distal joint. In addition, *T. occidentalis* is larger in size by about 60% - 70% for all measurements than the largest specimen of *T. bealensis* observed (Tables 2-1, 2-2). Examination of a number of other specimens of *T. bealensis* from other localities demonstrated that the *T. occidentalis* type specimen was well outside the range of intraspecific variation of *T. bealensis* (Table 2-3).

Sexual dimorphism within *T. bealensis* can be seen in the following structures: ovigers, gonopores, and cement gland. The ovigers of the females are half the size of males and bear short, elaborately forked terminal spines (Fig. 2-4E), whereas the male ovigers bear long, simply forked terminal spines (Figs. 2-4C, D). Gonopores of females are found on all walking legs, whereas male gonopores are only found on the last three pairs of walking legs (Fig. 2-5). In addition, female gonopores are approximately twice the size of male gonopores. Finally, the male cement gland is found on the femur of all walking legs, but is not present in females (Fig. 2-6).

The structure of the spines except heel, larger sole spines of the propodus and terminal spines of the oviger, were found to be hollowed tipped with bifurcated sides. King (1973) reports seeing hollow tipped spines in *Pycnogonum hancocki* and some members of the genus *Achelia*. These were particularly abundant on the palps in members of the genus *Achelia*. The function of the hollow tipped spines remains unknown, but similar structures in other arthropods have been thought to be chemosensory (King, 1973). This is the first report of spines with bifurcated sides, and their funcation remains unclear. While further examination of other pycnogonids is required, spines with hollow tips and bifurcated sides may become useful as another systematic character to help distinguish different pycnogonid groups.

The type specimen for *Tanystylum occidentalis* has suffered considerably over the years and now consists only of the appendages from the right side (chelifore, palp, ovigerous leg, and legs 1-3) mounted on a slide. The trunk, proboscis, left appendages, and the last pair of walking legs are missing. However, the type description (Cole 1904) is more than sufficient to fill in the gaps for most of the remaining characters although certain questions remain such as details of lateral process spination (Table 2-4). Information on these details will have to await collection of additional specimens of this species, known for now only from the type specimen. An attempt was made to collect at the type locality (Pacific Grove, California; May 2001), but neither suitable habitat nor additional specimens of *T. occidentalis* were found there.

In addition to the specimens of *Tanystylum bealensis* collected for this study, a number of museum specimens labeled *T. occidentalis* were examined (see list under Material Examined, Non-types) and found to belong to *T. bealensis* instead of *T. occidentalis*, leaving only the type specimen as the current sole representative of *T. occidentalis*.

Finally, a name change from *Tanystylum occidentalis* to *Tanystylum occidentale* is recommended in order to provide better gender agreement between the genus and species names in accordance with ICZN article 31.2 (International Commission on Zoological Nomenclature 1999).

2.3.7 Key to the ***Tanystylum*** *species (Ammotheidae) of the Pacific Coast of North America (Canada and United States, including Alaska)*

1 Chelifores one-segmented, achelate; three or four heel spines 2

1' Chelifores two-segmented, vestigial chelae or achelate; zero, two or three heel spines
.... 5

2(1) Proboscis conical with concave sides, distal portion an elongate tube; lateral processes with one or two dorsal tubercles; male gonopores not present on second coxa of second walking leg 3

2' Proboscis without concave sides and without an elongate tube in distal portion; lateral processes lacking dorsal tubercles; male gonopores present on second coxa of second walking leg 4

3(2) Ocular tubercle located at anterior edge of cephalic segment; medial proximal tubercle present on fourth palp segment; dorsal mounds on leg segments tibia one and two paired, lighter in color than remainder of body .... *T. californicum*

3' Ocular tubercle located in middle of cephalic segment; medial proximal tubercle absent on fourth palp segment; dorsal mounds on leg segments tibia one and two not paired, same color as remainder of body .... *T. duospinum*

4(2') Proboscis barrel-shaped, bluntly rounded at tip; chelifores joined at base; palps four-segmented, not extending past end of proboscis; abdomen without basal dorsal mound .... *T. occidentalis*

4' Proboscis conical with convex sides, straight at tip; chelifores separate at base; palps five-segmented, rarely four-segmented, extending past end of proboscis; abdomen with basal dorsal mound .... *T. bealensis* n.sp.

5(1') Palps five-segmented; presence of spines on lateral processes; two heel spines .... *T. nudum*

5' Palps six- or seven-segmented; presence or absence of spines on lateral processes; zero or three heel spines 6

6(5') Palps seven-segmented; three heel spines; trunk and legs covered with fine hair-like setae; abdomen orientated horizontally or ventrally; absence of spines on lateral processes .... *T. grossifemora*

6' Palps six- or seven-segmented; no distinct heel spines; no hair-like setae covering body or legs; abdomen orientated dorsally; presence of spines on lateral processes .... *T. intermedium*

2.4 Literature Cited

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Table 2-1. *Tanystylum bealensis* n.sp. (paratype; RBCM 002-00144-001) and *T. occidentalis* (holotype; USNM 1074913) multiple body measurements: trunk and right body appendages (excluding walking legs). Measurements given are in millimeters (mm) based on two duplicate measures; SE - standard error; n.a. – data not available.

	T. bealensis		*T. occidentalis*	
Structure	Mean	SE	Mean	SE
Trunk Length	0.806[b]	0.008	n.a.	n.a.
Trunk Width	0.660[b]	0.007	n.a.	n.a.
Abdomen	0.340[b]	0.003	n.a.	n.a.
Proboscis	0.371[b]	0.004	n.a.	n.a.
Palp, segment 1	0.048	0.002	0.080	0.002
Palp, segment 2	0.053	0.003	0.237	0.008
Palp, segment 3	0.165	0.003	0.059	0.001
Palp, segment 4	0.061	0.006	0.084	0.004
Palp, segment 5	0.120	0.003		
Oviger, segment 1	0.107[a]	0.002	0.160[a]	0.005
Oviger, segment 2	0.215	0.001	0.282[a]	0.013
Oviger, segment 3	0.109	0.003	0.208[a]	0.007
Oviger, segment 4	0.160	0.008	0.343[a]	0.001
Oviger, segment 5	0.218	0.002	0.338[a]	0.001
Oviger, segment 6	0.091	0.002	0.178[a]	0.006
Oviger, segment 7	0.078	0.005	0.121[a]	0.001
Oviger, segment 8	0.048	0.001	0.081[a]	0.001
Oviger, segment 9	0.052	0.001	0.085[a]	0.002
Oviger, segment 10	0.021	0.002	0.024[a]	0.002
Oviger, terminal spine	1.098	0.019	1.661[a]	0.001

[a] based on left appendage

[b] measured before specimen dissected

Table 2-2. *Tanystylum bealensis* n. sp. (paratype; RBCM 002-00144-001) and *T. occidentalis* (holotype; USNM 1074913) multiple body measurements: right walking legs. Measurements are in millimeters (mm) based on two duplicate measures; SE - standard error; n.a. – data not available.

	T. bealensis		*T. occidentalis*			*T. bealensis*		*T. occidentalis*	
Structure	Mean	SE	Mean	SE	Structure	Mean	SE	Mean	SE
1st leg, coxa 1	0.167	0.012	0.246	0.001	3rd leg, coxa 1	0.173	0.001	0.241	0.002
1st leg, coxa 2	0.219	0.016	0.380	0.042	3rd leg, coxa 2	0.214	0.002	0.312	0.003
1st leg, coxa 3	0.211	0.016	0.275	0.008	3rd leg, coxa 3	0.183	0.007	0.241	0.014
1st leg, femur	0.505	0.008	0.861	0.002	3rd leg, femur	0.420	0.002	0.667	0.014
1st leg, tibia 1	0.463	0.005	0.742	0.013	3rd leg, tibia 1	0.394	0.002	0.608	0.011
1st leg, tibia 2	0.500	0.004	0.829	0.013	3rd leg, tibia 2	0.453	0.003	0.672	0.001
1st leg, tarsus	0.065[a]	0.000	0.046	0.003	3rd leg, tarsus	0.094	0.010	0.082	0.001
1st leg, propodus	0.302[a]	0.003	0.542	0.005	3rd leg, propodus	0.356	0.001	0.496	0.010
1st leg, main claw	0.175[a]	0.001	0.259	0.012	3rd leg, main claw	0.217	0.004	0.306	0.009
1st leg, aux claw	0.092[a]	0.001	0.146	0.001	3rd leg, aux claw	0.095	0.005	0.177	0.018
2nd leg, coxa 1	0.161	0.008	n.a.	n.a.	4th leg, coxa 1	0.139	0.004	n.a.	n.a.
2nd leg, coxa 2	0.243	0.002	0.289	0.001	4th leg, coxa 2	0.217	0.007	n.a.	n.a.
2nd leg, coxa 3	0.183	0.008	0.288	0.019	4th leg, coxa 3	0.187	0.004	n.a.	n.a.
2nd leg, femur	0.463	0.008	0.695	0.001	4th leg, femur	0.370	0.003	n.a.	n.a.
2nd leg, tibia 1	0.395	0.006	0.635	0.008	4th leg, tibia 1	0.342	0.002	n.a.	n.a.
2nd leg, tibia 2	0.491	0.019	0.691	0.007	4th leg, tibia 2	0.407	0.007	n.a.	n.a.
2nd leg, tarsus	0.089	0.007	0.085	0.001	4th leg, tarsus	0.082	0.001	n.a.	n.a.
2nd leg, propodus	0.359	0.001	0.499	0.004	4th leg, propodus	0.347	0.000	n.a.	n.a.
2nd leg, main claw	0.208	0.002	0.283	0.002	4th leg, main claw	0.200	0.005	n.a.	n.a.
2nd leg, aux claw	0.083	0.002	0.174	0.011	4th leg, aux claw	0.099	0.004	n.a.	n.a.

[a] based on left appendage

Table 2-3. Comparision of intraspecific variation in *Tanystylum bealensis* n.sp. with *T. occidentalis* (holotype; USNM 1074913) for non-dissected characteristics. Measurements given are in millimeters (mm); SD - standard deviation; N - number of specimens; n.a. – data not available.

	T. bealensis			*T. occidentalis*
Structure	Mean	SD	N	
Trunk Length	0.836	0.064	10	n.a.
Trunk Width	0.681	0.051	10	n.a.
Abdomen	0.320	0.024	10	n.a.
Proboscis	0.405	0.032	10	n.a.
Palp, segment 5	0.146	0.011	10	n.a.
Oviger, segment 4	0.183	0.026	10	$0.343^{a,b}$
Oviger, segment 5	0.221	0.022	10	$0.338^{a,b}$
3rd Right Leg				
Femur	0.475	0.048	10	0.667^{b}
Tibia 1	0.458	0.054	10	0.608^{b}
Tibia 2	0.459	0.051	10	0.672^{b}

[a] based on left appendage

[b] significantly different; unpaired 2-tailed t-test; $p<0.05$

Table 2-4. Comparision of characters between *Tanystylum bealensis,* n. sp. and *Tanystylum occidentalis* (Cole, 1904).

Character	*Tanystylum bealensis*	*Tanystylum occidentalis*
Spination on lateral processes	typically two or three, one on fourth pair	Unknown (none mentioned)[a]
Basal abdomen shape	dorsal mound present	dorsal mound absent[a]
Abdomen spination	base with five spines, tip with six spines	two or three short stiff spines on dorsal tip[a]
Collar at proboscis base	present	absent[a]
Proboscis shape	slightly conical, tapering with convex sides towards mouth, with a straight end	barrel-shape with round end[a]
Number of palp segments	five, very rarely four	four[b]
Length of palps	extend past end of proboscis	do not extend past end of proboscis[a]
Arrangement of spines on terminal palp segment	present on ventral, lateral and distal surfaces	present only on distal surface[b]
Chelifores connected at base	no	yes[a]
Number of spines on chelifore	four dorso-distal spines	two or three spines distally, one orientated ventrally[b]
Shape of male oviger terminal spines	elongate, simple forked	elongate; appears unforked, possibly simply forked[b]
Tarsus spination	eight short spines on ventro-distal edge and one to three short spines proximally in parallel orientation	one large, stout spine on ventral surface with two or three smaller spines[b]
Position of cement gland on femur of males	three-quarters along femur from proximal joint, between distal row of spines and middle spines on dorsal surface	at distal joint, directly behind distal row of spines[b]

[a] From type description (Cole, 1904)
[b] From holotype

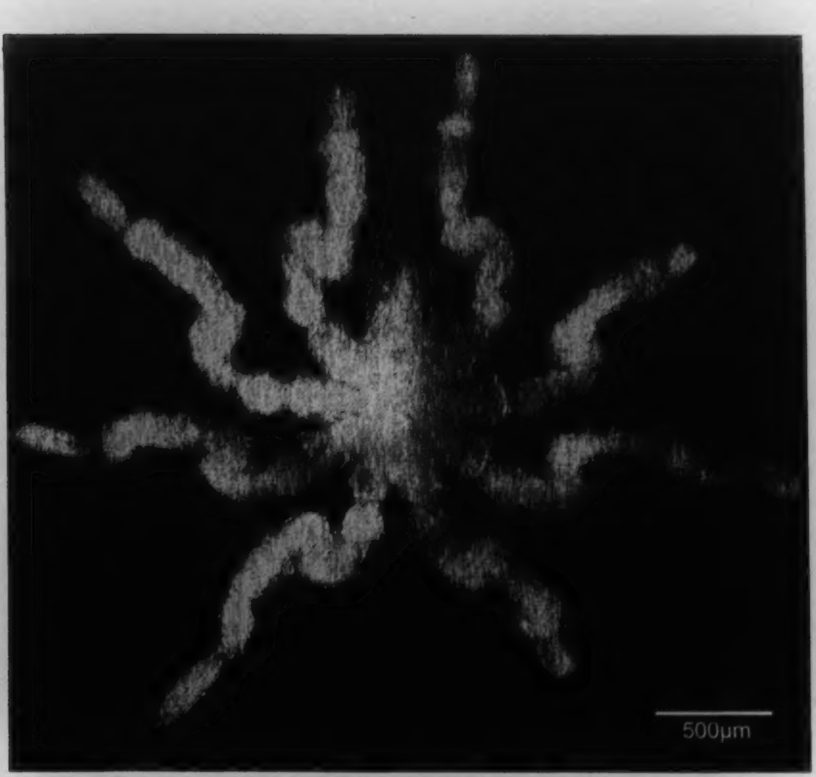

Figure 2-1. Dorsal view of *Tanystylum bealensis* n.sp., male holotype, collected from Cape Beale, Barkley Sound, British Columbia (USNM XXXXXXX).

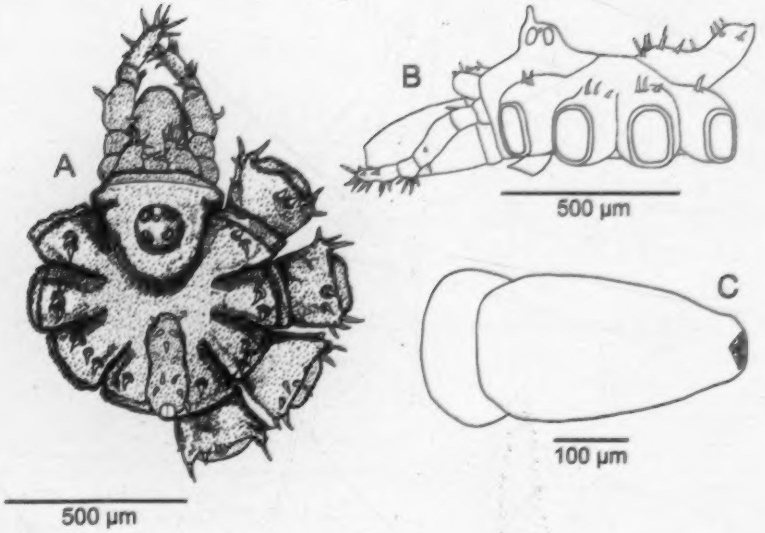
B
A
500 µm
C
100 µm
500 µm

Figure 2-2. *Tanystylum bealensis* n.sp., male: A, trunk, dorsal view with legs removed; B, trunk, left lateral view with legs removed; C, proboscis, ventral view.

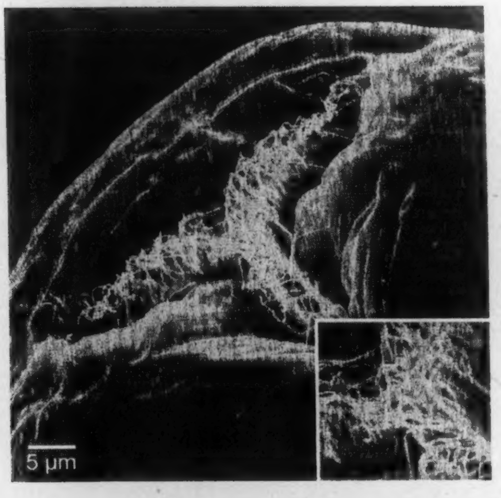

Figure 2-3. Proboscis tip of *Tanystylum bealensis* n.sp., male, showing the triradiate mouth ringed with hair-like projections along inner edges of lips. Inset: an enlargement of the mouth showing the hair-like projections.

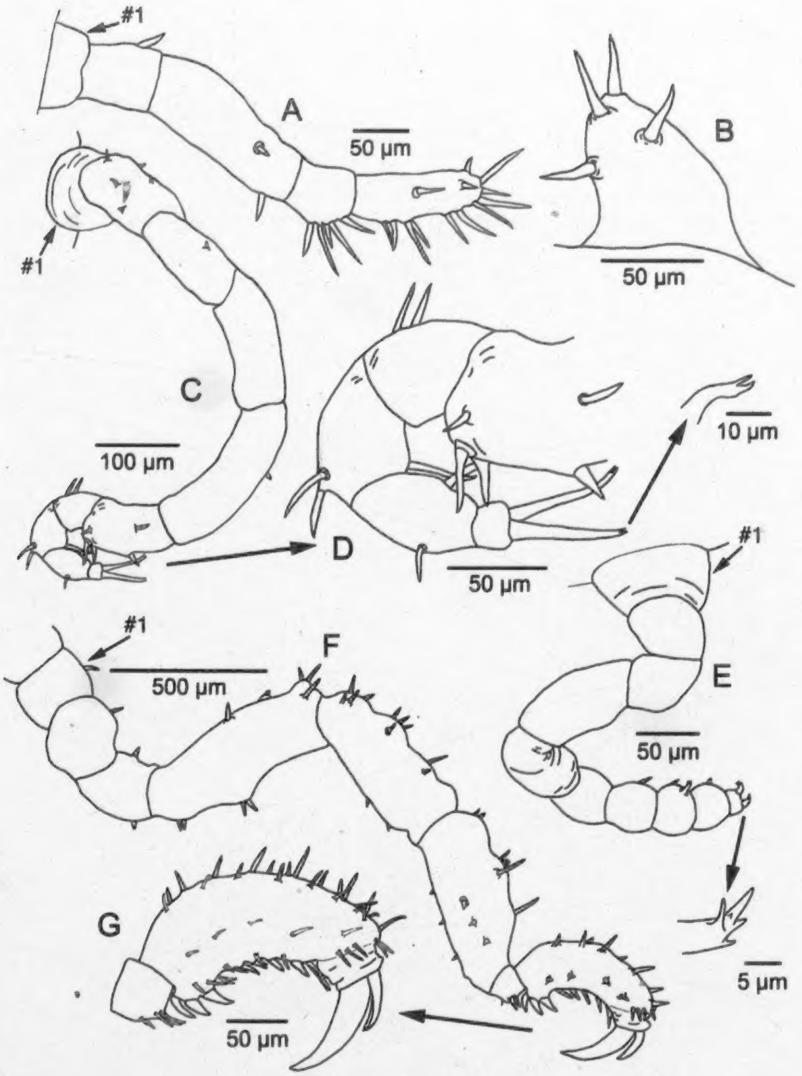
#1
A
50 µm
B
#1
50 µm
C
100 µm
10 µm
D
50 µm
#1
#1
F
500 µm
E
50 µm
G
5 µm
50 µm

Figure 2-4. *Tanystylum bealensis* n.sp., male: A, palp, right lateral view; B, chelifore, dorsal view; C, right oviger, ventral view; D, right oviger, ventral view of terminal segments, enlarged; E, right female oviger, lateral view; F, third right walking leg; G, terminal segments of third right walking leg, enlarged.

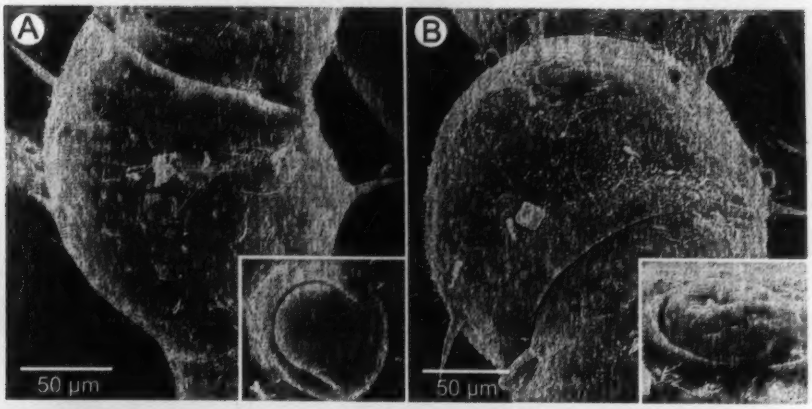

Figure 2-5. Gonopores of *Tanystylum bealensis* n.sp. from the second coxae of the second right walking leg with an inset illustrating an enlargement of the gonopore from the middle of each coxal segment. A, male; B, female.

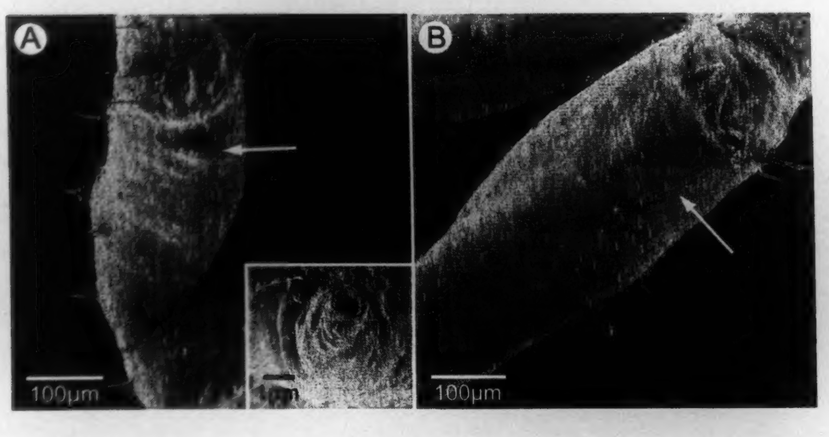

Figure 2-6. Cement gland of *Tanystylum bealensis* n.sp. on the dorsal surface of the femur of the third right walking leg. A, male; B, female (absent). Inset illustrating an enlargement of the male cement gland spine.

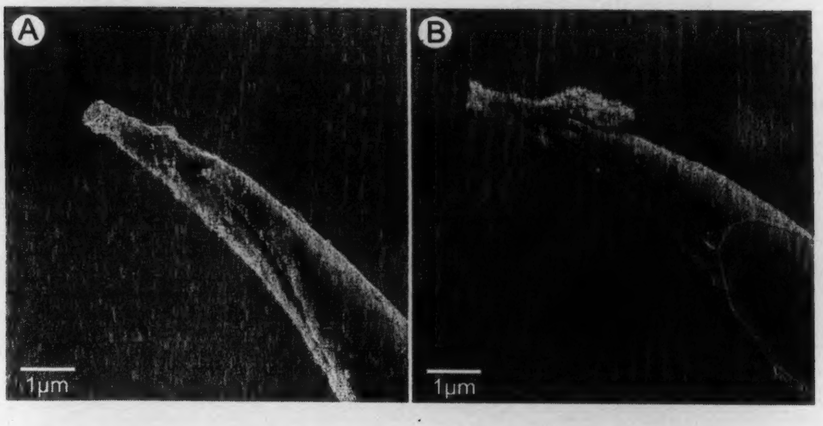

Figure 2-7. Spines of *Tanystylum bealensis* n.sp. illustrating the hollow tips and bifurcated sides. A, on fourth walking leg; B, on chelifore.

CHAPTER THREE*

Postembryonic Development of *Tanystylum bealensis* (Pycnogonida, Ammotheidae) from Barkley Sound, British Columbia, Canada

3.1 Introduction

The development of pycnogonids (sea spiders) may provide key insights into their evolution and relations to other chelicerate and arthropod groups. By further understanding instars in pycnogonids we are expanding possible identification methods for new and existing species. Furthermore, by correctly identifying a new or current species, we can then potentially start to resolve many of the confusing relationships found within this engimatic group. Pycnogonids or sea spiders (Phylum Arthropoda, Subphylum Chelicerata, Class Pycnogonida) are a small group of marine chelicerates found worldwide in many different habitats ranging from intertidal to abyssal depths (Hedgpeth 1947; King 1973; Arnaud and Bamber 1987). They are unique among all marine invertebrates because of their exclusive paternal care (Ridley 1978; Ghiselin 1987), with adult males carrying the eggs and, in some cases, larvae, on their ovigers (King 1973; Nakamura and Sekiguchi 1980; Arnaud and Bamber 1987; Bain 2003).

Pycnogonid development can be divided into two aspects, embryonic and post-embryonic. Embryonic includes the time from fertilization until larval hatching. Postembryonic encompasses development form hatchling to adult. Postembryonic development in pycnogonids has recently been summarized by Bain (2003), where four different types are described: the Typical Protonymphon (Morgan 1891; Okuda 1940; Bain 2003), the Encysted Larva (Hilton 1916; Lebour 1945; Staples and Watson 1987; Bain 2003), the Atypical Protonymphon (Ohshima 1933 1937; Salazar-Vallejo and Stock 1987; Bain 2003), and the Attaching Larva (Sekiguchi et al. 1971; Nakamura 1981). The Typical Protonymphon, the Encysted Larva, and the Atypical Protonymphon all hatch as a protonymphon (a free-living, feeding stage) and then, depending on species, follow one of three different developmental pathways. The Typical Protonymphon, the most common developmental type, remains free-living and undergoes a series of molts during which it increases in size, modifies its larval appendages into adult palps and ovigers and

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* This chapter has been co-authored with Dr. Bonnie Bain and is being prepared for submission to the journal 'Journal of Morphology'.

adds walking legs in a sequential fashion beginning with limb buds which form at the posterior end of the larva (Morgan 1891; Okuda 1940). The Encysted Larva, restricted to the Family Phoxichilidiidae and one species of the Family Ammotheidae, burrows into a hydroid or coral polyp and undergoes a similar series of molts, but with fewer stages (Hilton 1916; Lebour 1945; Staples and Watson 1987; Russel and Hedgpeth 1990). The Atypical Protonymphon, found only in a few genera of the Family Ammotheidae, either burrows into a clam or attaches to the outside of a nudibranch or a polychaete and then undergoes a modified series of molts in which all the adult appendages appear at once as limb buds and then, at each subsequent molt, add more segments until the adult number is reached (Ohshima 1933, 1937; Salazar-Vallejo and Stock 1987).

All pycnogonid stages, whether a juvenile or an adult, must molt to grow. In *Pycnogonum littorale*, adults continue to molt after having reached sexual maturity, with each leg shed separately as a sleeve, and lateral splits in the body cuticle that allow it to be shed as a dorsal and ventral piece (King 1973; Arnaud and Bamber 1987).

There are very few published descriptions of complete pycnogonid life cycles (see summary in Bain 2003), and of these only two, for *Propallene longiceps* (Attaching Larva) and *Pycnogonum litorale* (very highly modified Typical Protonymphon), are known in sufficient detail because these two species were lab-reared with all stages collected and described. The two most complete series of postembryonic developmental stages from field collections are for *Tanystylum orbiculare*, described by Morgan (1891) and *Achelia alaskensis*, described by Okuda (1940) and both of these have the Typical Protonymphon type of development. Morgan (1891) described ten stages (protonymphon-adult) that had been collected from hydroids, but was uncertain whether or not he had collected all life history stages. Okuda (1940) described eight developmental stages that had been found on *Polyorchis karafutoensis* medusae. The first stage was an advanced protonymphon (in which the posterior region has begun to elongate) and the last stage was a juvenile with a seven-segmented oviger.

During the summer of 1999, a large number of *Tanystylum bealensis* (Typical Protonymphon), including all life history stages, were collected in Barkley Sound, British Columbia, Canada. The specimens collected (n= 2397) contained many replicates from each of the life history stages of this species, thus allowing for the first complete description of all life stages from a natural population.

3.2 Materials and Methods

Specimens of *Tanystylum bealensis* Gillespie and Bain, 2004 were collected from the exposed side of Cape Beale, Barkley Sound, British Columbia, Canada (48° 47'N 125° 13'W). This species was found feeding on colonies of the hydrozoan *Plumularia setacea* (Ellis, 1755), which were attached to mussels (*Mytilus californianus* Conrad, 1837) in the lower intertidal zone (below 0.6m tidal height). All instars except the first (protonymphon) were found free living on the hydroid *Plumularia setacea*. Instar one was found attached to the ovigers of the adult male (Fig. 1-1). The mussels were collected and shucked to prevent dilution of the fixative. The shells of each mussel, which were covered with hydroids and unsorted pycnogonids, were then all fixed together in 10% formalin in seawater for later analysis.

Specimens were separated from the hydroid and sorted into different instars using a dissecting microscope at 60X magnification. Trunk length (from chelifore insertion to tip of fourth lateral processes) was measured using a calibrated ocular micrometer for each instar and egg diameter was measured externally on male ovigers. Specimens for the SEM were run through a chemical dehydration series, first in a graded ethanol series (15%, 25%, 40%, 50%, 70%, 80%, 95% 100%) and then through a hexamethyldisilzane (HMDS) graded series (1:1 of 100% ethanol and HMDS, then 100% HMDS). They were then sputter-coated with gold using an SEM coating system (SEM PREP 2), and viewed with a Hitachi S450 scanning EM at 10-20 kV. All drawings were made from SEM images.

3.3 Results

3.3.1 First Instar or Protonymphon (Figs. 3-1, 3-2A, 3-3A, 3-4A, 3-5A)

The first instars (n =16) were always found either on the male's ovigers or on the remaining unhatched eggs (Fig. 3-1) and have a roughly oval-shaped trunk (Fig. 3-2A) (trunk length: 0.167 mm +/- 0.004 (SE)), a pair of large, two-segmented chelifores (Fig. 3-3A) and two pairs of larval appendages (Figs. 3-3A, 3-4A, 3-5A). The ventral surface of the chelifore scape bears a single, stout spine (Figs. 3-2A, 3-3A) and the two pairs of larval appendages (the future palps and ovigers) are each two-segmented with a long terminal spine and the first segment of each have a lateral spine that extends approximately half the length of the second segment (Figs. 3-4A, 3-5A). The proboscis (Fig. 3-2A) is nearly as long as the chelifore scape at this stage and the mouth is trimeric, formed by three bare lips at the tip of the proboscis.

3.3.2 Second Instar (Figs. 3-2B, 3-3B, 3-4B, 3-5B)

The second instar (n = 70) (and all subsequent instars) are found on the hydroid *Plumularia setacea.* The body is nearly double the size (trunk length: 0.294 mm +/- 0.003 (SE)) of the previous instar and the posterior region has begun to elongate (Fig. 3-2B) and form the first pair of limb buds. The chelifores are unchanged except that one dorsal and one lateral spine have appeared on each scape (Fig. 3-3B) and the two pairs of larval appendages are slightly larger while the spine on the first segment of each appears to have shrunk (Figs. 3-4B, 3-5B). The proboscis has shortened to only 2/3 the length of the chelifore scape (Fig. 3-2B).

3.3.3 Third Instar (Figs. 3-2C, 3-3C, 3-4C, 3-5C)

This instar (n = 199) is much larger than the previous one (trunk length: 0.418 mm +/- 0.003 (SE)) and a single pair of limb buds has formed on the posterior region of the trunk (Fig. 3-2C). Each limb bud has one terminal spine and one small lateral spine. The abdomen has now formed and is located in between the pair of limb buds (Fig. 3-2C). The chelifores (Fig. 3-3C) and the two pairs of larval appendages (Figs. 3-4C, 3-5C) are relatively unchanged.

3.3.4 Fourth Instar (Figs. 3-2D, 3-3D, 3-4D, 3-5D, 3-6A)

The first pair of walking legs have appeared (n = 240; trunk length: 0.556 mm +/- 0.002 (SE)) and a pair of limb buds, which will become the second pair of walking legs in the next instar, have formed behind the first pair of walking legs (Fig. 3-2D). The first pair of walking legs each have six segments: coxae 1-3, femur, tibia, propodus and, at this stage, are missing the second tibia and the tarsus (Fig. 3-6A). The propodus has a single heel spine and three sole spines, the main claw is the same length as the propodus, and the auxiliary claws are 1/16 the length of the main claw. The chelifores (Fig. 3-3D), larval appendages (Figs. 3-4D, 3-5D), and the proboscis (Fig. 3-2D) are unchanged from the previous instar except that it has continued to shrink relative to body size. The anus has opened at the tip of the abdomen (Fig. 3-2D).

3.3.5 Fifth Instar (Figs. 3-2E, 3-3E, 3-4E, 3-5E, 3-6B, 3-7A)

The fifth instar (n=398; trunk length: 0.614 mm +/- 0.002 (SE)) has two pairs of walking legs and a pair of limb buds (Fig. 3-2E). The first pair of walking legs, six-segmented in the previous instar, now have the adult number of segments (eight), having added the additional tibia and tarsus. The propodus has added two more sole spines, and the auxiliary claws are now 1/6 the length of the main claw (Fig. 3-6B). The second pair of walking legs has six segments, missing a tibia and the tarsus (Fig. 3-7A).

3.3.6 Sixth Instar (Figs. 3-2F, 3-3F, 3-4F, 3-5F, 3-6C, 3-7B, 3-8A)

The sixth instar (n = 337; trunk length: 0.770 mm +/- 0.003 (SE)) has three pairs of walking legs and a pair of limb buds which will become the fourth pair of walking legs in the next instar (Fig. 3-2F). The tarsus of the first pair of walking legs has added three ventral spines and the propodus has added an additional heel spine and one more sole spine (for a total of six sole spines) (Fig. 3-6C). The second pair of walking legs has become eight-segmented with the addition of a tibia and the tarsus, following the same pattern as the first pair of walking legs (Fig. 3-7B). The third pair of walking legs are six-segmented, missing a tibia and the tarsus (Fig. 3-8A).

The chelifores (Fig. 3-3F) and the first pair of larval appendages (the future palps) (Fig. 3-4F) are relatively unchanged in this instar, but the second pair of larval appendages (the future ovigers) undergo a radical change, each losing most of their structure and being reduced to a very small unsegmented knob (Figs. 3-2F, 3-5F). The beginnings of the ocular tubercle (Fig. 3-2F), complete with four red eyes, have appeared. This is the first instar in which the eyes are visible. The ocular tubercle is a small rounded mound and the eyes are spread equidistant around it. The abdomen has become more elongate and a pair of dorsal spines have formed near the tip (Fig. 3-2F).

3.3.7 Seventh Instar (Figs. 3-2G, 3-3G, 3-4G, 3-5G, 3-6D, 3-7C, 3-8B, 3-9A)

The seventh instar (n = 368; trunk length: 0.825 mm +/- 0.002 (SE)) has four pairs of walking legs (Fig. 3-2G) and all except the last pair have the adult number of segments (eight) (Figs. 3-6D, 3-7C, 3-8B). The fourth pair is six-segmented at this stage, again missing a tibia and the tarsus (Fig. 3-9A). The first pair has added two additional tarsal and two additional propodal sole spines, the main claw is now 3/4 the length of the propodus, and the auxiliary claws are 1/4 the length of the main claw (Fig. 3-6D).

The chelifores (Fig. 3-3G) are again relatively unchanged at this stage, but the first pair of larval appendages have lost the long terminal spines and have developed in their place a number of smaller spines (Fig. 3-4G). The ovigers, remnants of larval appendage two in the previous instar, have increased in size, but remain unsegmented (Figs. 3-2G, 3-5G). The ocular tubercle is much larger and has gone from a low mound to a large truncated cone for a base, topped by a much smaller cone (Fig. 3-2G). The abdomen, horizontal in previous instars, now points at an angle (dorsal-anteriorly) and, in length, it reaches to the beginning of the third coxa of the fourth pair of walking legs (Fig. 3-2G).

3.3.8 Eighth Instar (Figs. 3-2H, 3-3H, 3-4H, 3-5H, 3-6E, 3-7D, 3-8C, 3-9B)

The eighth instar (n = 312; trunk length: 0.868 mm +/- 0.002 (SE)) (Fig. 3-2H) has begun to resemble the adult in certain features: all four pairs of walking legs now have their full complement of segments (Figs. 3-6E, 3-7D, 3-8C, 3-9B), the chelifores are no longer chelate (Fig. 3-3H), and the palps have the adult number of segments (five, or rarely four (<1%)) (Fig. 3-4H). In other respects, it is still a juvenile. The tarsal and propodal spines have not yet reached their adult number and arrangement. For instance, the first walking leg tarsus of the eighth instar has only one spine in its second tarsal row (Fig. 3-6E) instead of three in the adults of this species (Figs. 3-6F, 3-6G) and the adult propodus has three heel spines and many more sole spines (Figs. 3-6F, 3-6G) than the eighth instar (Fig. 3-6E).

The ovigers, which were a single segment in the previous instar, have become six-segmented in this instar (Figs. 3-2H, 3-5H). Although always six-segmented, size varies considerably among eighth instar larvae (up to 2x). The upper cone of the ocular tubercle has doubled in size and the four red eyes occupy the majority of the space in the lower cone (Fig. 3-2H). The abdomen has shrunk in size relative to the body and now only reaches to the base of the second coxa of the fourth pair of walking legs while the proboscis length has increased in size to one half the trunk length (Fig. 3-2H). The three lips around the mouth are now covered with hair-like setae.

3.3.9 Ninth Instar or Adult (Figs. 3-2IJ, 3-3IJ, 3-4IJ, 3-5IJ, 3-6FG, 3-7EF, 3-8DE, 3-9CD)

Tanystylum bealensis adults (females, n = 213, trunk length: 0.993 mm +/- 0.005 (SE); males, n = 244, trunk length: 1.007 mm +/- 0.005 (SE)) exhibit a number of changes from the previous instar. All four pairs of walking legs now have their adult complement of tarsal spines (eight ventro-distal spines and one to three mid-ventral spines) and propodal spines (three heel spines and 13 sole spines in legs one-three; three heel spines and only nine sole spines in leg four), the main propodal claw is half the length of the propodus, and the auxiliary claws are half the length of the main claw (Fig. 3-6FG). The chelifores have added four dorso-distal spines (Fig. 3-3IJ) and the palps have the following adult spination: segment two with a single dorsal spine, segment three with a single distal lateral spine and a single distal ventral spine, segment four with a cluster of four to five ventral spines, segment five with two mid-lateral spines, three small clusters of ventral spines, and four to five terminal spines (Fig. 3-4IJ).

The ovigers have become ten-segmented in both males and females (Fig. 3-5IJ) and the male ovigers are twice the size of the female ones. Segments one to six of the female oviger lack spines. Segments seven to nine each have a pair of short ventral spines, and segment ten has a pair of short terminal spines, each of which has four points at its tip (Gillespie and Bain 2004). The first segment of the male oviger is without spines, segment two has four to six scattered spines, segment three has one dorso-distal spine, segments four and five each have one or two scattered spines, segment six has three mid-ventral spines and four ventro-distal ones, segments seven and eight each have a pair of dorso-distal spines and segment eight also has an additional ventro-distal spine, segment nine has two dorso-lateral spines, one on either side of the segment, and segment ten has a pair of very long double-pointed terminal spines (Gillespie and Bain 2004).

The upper cone of the ocular tubercle has doubled the size of instar eight and the abdomen exhibits a degree of sexual dimorphism (Fig. 3-2IJ). The female abdomen (Fig. 3-2I) extends to the base of the second coxa of the fourth leg while in most males the abdomen (Fig. 3-2J) is typically shorter, extending only over the first coxa of the fourth legs, but a few males have a longer abdomen than the females. The adult abdomen, in both sexes, has more spines. The tip now has five or six spines, instead of two as in the previous instar and the base of the abdomen, where it connects with the trunk, has five or six spines. The proboscis has increased in size to 3/4 the trunk length and the lateral processes (located at the base of each walking leg) have added more dorso-distal spines. The first three pairs of lateral processes have two or three spines and the fourth pair has one to three spines with one spine the most common state.

The female gonopores are located on the ventral side of the second coxae of all four pairs of walking legs. The male gonopores are in the same location, but are only found on the last three pairs of walking legs. The cement gland (male only) is a single dorso-distal pore located directly behind the group of dorsal spines at the distal end of the femur on all four pairs of walking legs. Eggs were found on the base of the adult male's ovigerous legs in bunches of approximately 40 (Fig. 3-1). Egg diameter measured externally on the male oviger was 0.068um +/- 0.001 (SE; n=12).

3.4 Discussion

The postembryonic development of *Tanystylum bealensis* is characterized by the protonymphon larvae having three pairs of appendages, the chelifores and two pairs of larval appendages that are lost or modified over several molts. The larvae also only remain on the adult male during the first instar, after which they are found on a hydroid (in this case, *Plumularia setacea*). Finally the larvae acquire the paired walking leg limb buds individually over a period of several molts (see below). This mode of postembryonic development (Typical Protonymphon) is shared by all species in the genus *Tanystylum*, in the closely related genus *Achelia*, and in the more distantly related *Pycnogonum* where larval development is known (Morgan 1891; Okuda 1940; Tomaschko et al 1997; Bain 2003).

Walking leg development in *Tanystylum bealensis* follows a specific pattern. In walking leg one, limb buds first appear in the third instar, are changed to walking legs with six segments (coxae 1-3, femur, tibia, propodus) in instar 4, and finally in the fifth instar, the six-segmented walking legs become eight-segmented in the following manner: the femur splits into two, forming a new femur and an additional tibia (Morgan 1891; Bain 2003) and the propodus splits, forming the tarsus-propodus (Bain 2003) (Table 3-1). The remaining three walking legs follow the same pattern of leg development as the first walking leg, except the limb buds appear only one instar before the corresponding walking leg, as opposed to appearing two instars earlier. This pattern of leg development has also been described (Morgan 1891) for the closely related species, *Tanystylum orbiculare*, but it differs radically from the mode of development of leg segmentation described by Okuda (1940) for the more distantly related *Achelia alaskensis* (Table 3-1).

Postembryonic development in *Tanystylum bealensis* differs from that of *T. orbiculare* in several respects. *T. bealensis* undergoes a total of nine molts (instars) from egg to adult while *T. orbiculare* is described as having at least ten stages (Morgan 1891; Bain 2003) with an extra stage in *T. orbiculare* (Stage 2 in Morgan 1891) found between the first and second instars of *T. bealensis*. In *T. bealensis*, the first instar remains on the adult male for a short time after hatching while all individuals of the first stage of *T. orbiculare* were found living on a bed of hydroids. Instars three through seven are very similar in these two species, but more differences appear in the eighth instar: both the chelifores (one segment in *T. bealensis*, two segments in *T. orbiculare*) and the palps (five segments in *T. bealensis* and six in *T. orbiculare*) acquire their adult number of segments and the ovigers but, while very similar in overall appearance in both species, are six-segmented in *T. bealensis* and appear to be unsegmented in *T. orbiculare*. The apparent differences in development between these two species may result from several factors. First, they may be differences related to timing and development differences between these two species. Second, they may be due to problems such as inadequate sampling of populations or artifacts due to preservation and slide preparation. For instance, of the two species, only *T. bealensis* is known to stay on the parent for a period of time after emerging from the egg, *T. orbiculare* may do likewise, but this may not have been observed in the earlier study (Morgan 1891). The extra instar (between the first and second instars of *T. bealensis*) in *T. orbiculare* may be an artifact based on incomplete knowledge of both life cycles or it may be an additional instar that has not yet been documented in *T. bealensis* since the extra stage of *T. orbiculare* was based on internal structures, which have not yet been examined for *T. bealensis*. However, since the extra stage of *T. orbiculare* is based on a single specimen and there are no external differences between the extra stage (Stage 2 in Morgan 1891) and stage 1 specimens, it is probable that this extra stage is a larger specimen of the same stage or instar. This would indicate that both specimens of *Tanystylum* have a total of nine molts (instars) from egg to adult. Most of the differences that appear in the eighth instar are due to differences between the two species. However, apparent lack of segmentation in the ovigers of *T. orbiculare* at this stage may be an artifact due to problems with slide preparation and inadequate microscope resolution since in overall appearance, the ovigers of this species seem to be identical to the condition found in *T. bealensis* at this stage.

The most thorough description of the post-embryonic development of an *Achelia* species has been done by Okuda (1940) for *Achelia alaskensis*. Comparison of the post-embryonic development of *T. bealensis* with *A. alaskensis* suggests possible developmental differences between these two genera. Unlike *T. bealensis* and *T. orbiculare*, the larvae of *Achelia alaskensis* are found on the medusa of *Polyorchis karafutoensis*, under the exumbrella and on the wall of the manubrium. The earliest stage (stage 1) found by Okuda (1940) corresponds to instar two of *T. bealensis*. Although habitat is different between the three species at this point in development, all larvae are free-living. Not until instar five (stage 4 of Okuda (1940), are differences observed between the two genera. In *A. alaskensis*, the chelae now bear three teeth, and the chelifore cement spine is reduced, whereas the two *Tanystylum* species do not have teeth on the chelae and do not show a reduced of the chelifore cement spine until the next instar. In addition, there is also a difference in the number of segments of the second walking leg, which has appeared for the first time in instar five (Table 3-1). In *Tanystylum*, it is always six segmented, missing tibia 1 and the tarsus, and becoming eight segmented in the next instar. In *A. alaskensis* however, the second walking leg is initially six segmented, but later becomes seven segmented, during the same stage, with the differentiation of the fourth segment into the femur and the first tibia. Instar six and seven show the same pattern in the emergence of walking legs three and four between the two genera (Table 3-1). In *Tanystylum*, both legs are initially six-segmented, becoming eight-segmented in the next molt. In *A. alaskensis*, the third walking leg emerges seven-segmented, while the fourth walking leg emerges with an adult form of eight segments. The final stage (stage seven) collected by Okuda (1940) corresponds to instar 8 of *T. bealensis*. The chelifores of *Tanystylum* have become achelate and one- (*T. bealensis*) or two-segmented (*T. orbiculare*), whereas *A. alaskensis* retains its fully functionally chelae. While the palps in both genera increase to the number of segments seen in the adults, only *Tanystylum* increases to five- (*T. bealensis*) or six-segmented (*T. orbiculare*) palps, while *A. alaskensis* increases to eight-segmented palps. The ovigerous legs of both genera also increase from a single segment (instar seven) to six-segments, but somehow *A. alaskensis* adds an additional segment and a terminal spine later in the instar. While no further stages were available, *A. alaskensis* would likely follow a similar path in development with one or two additional instars to reach maturity as the adult. This would result in the reduction of the chelifores to achelate and two-segmented, the addition of three segments to the ovigerous legs, bringing it up to ten-segments, and the development of gonopores. However, until the complete postembryonic development is described for an *Achelia* species, the total number of instars from egg to adult will remain uncertain and the development described by Okuda (1940) for *A. alaskensis* cannot be judged as characteristic for the entire genus *Achelia*.

Although this post-embryonic description was not used in the species description of *T. bealensis*, several interesting differences were found between *T. bealensis, T. orbiculare* and *A. alaskensis*. First is the appearance of walking legs, which is similar between both *T. bealensis* and *T. orbiculare* but markedly different than *A. alaskensis*. Secondly, the number of instars in the *Tanystylum* species is nine while *A. alaskensis* has ten instars. The development of pycnogonids may provide information relating to their phylogenetic relationships (Nakamura 1981). This information, though lacking in large sample comparisons between and within genera, may provide taxonomic indicators at the genus level. However, to examine the possibility of using post-embryonic development as an additional characteristic for the diagnosis of a genus, the post-embryonic development of the closely related genus, *Achelia*, needs to be further examined. Additionally, by understanding the key characteristics of each instar we can better identify other instar specimens that often sit unidentified in many museum collections.

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Table 3-1. Comparison of the postembryonic development of the walking legs in *Tanystylum bealensis* (Gillespie and Bain, 2004), *Tanystylum orbiculare* (Morgan, 1891) and *Achelia alaskensis* (Okuda, 1940). Table modified from Nakamura (1981).

Larval Stage of *T. bealensis*	Number of walking legs (pairs)	Number of segments	Larval Stage of *T.orbiculare*	Number of walking legs (pairs)	Number of segments	Larval Stage of *A. alaskensis*	Number of walking legs (pairs)	Number of segments
Instar 2	0[1]		Stage 3	0[1]		Stage 1	0[1]	
Instar 3	0[1]		Stage 4	0[1]		Stage 2	0[1]	
Instar 4	1[2]	6[a]	Stage 5	1[2]	6[a]	Stage 3	1[2]	6[a]
Instar 5	2[3] 1st	8	Stage 6	2[3] 1st	8	Stage 4	2[3] 1st	8
	2nd	6[a]		2nd	6[a]		2nd	6[a] or 7[b]
Instar 6	3[4] 1st	8	Stage 7	3[4] 1st	8	Stage 5	3[4] 1st	8
	2nd	8		2nd	8		2nd	8
	3rd	6[a]		3rd	6[a]		3rd	7[b]
Instar 7	4 1st	8	Stage 8	4 1st	8	Stage 6	4 1st	8
	2nd	8		2nd	8		2nd	8
	3rd	8		3rd	8		3rd	8
	4th	6[a]		4th	6[a]		4th	8
Instar 8	4 1st	8	Stage 9	4 1st	8	Stage 7	4 1st	8
	2nd	8		2nd	8		2nd	8
	3rd	8		3rd	8		3rd	8
	4th	8		4th	8		4th	8

[1] first limb buds present

[2] second limb buds present

[3] third limb buds present

[4] four limb buds present

[a] missing tibia one and tarsus

[b] tarsus present, but missing a tibia

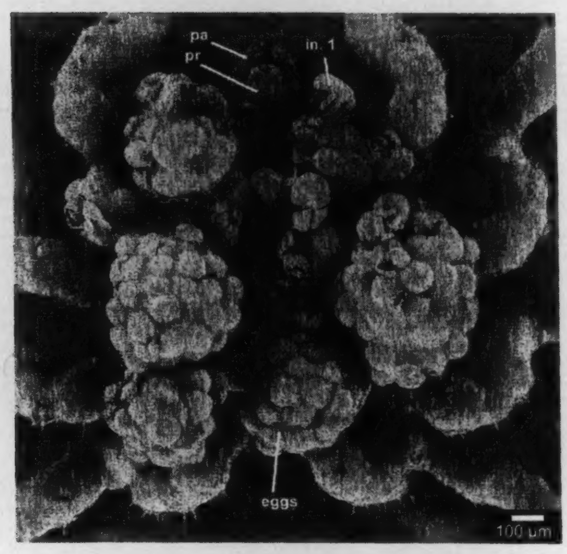

Figure 3-1. *Tanystylum bealensis* male, ventral view, showing eggs and instar 1 (protonymphon) on ovigerous legs. in. 1, instar 1 (protonymphon); pa, palps; pr, proboscis; 1, first walking leg; 2, second walking leg; 3, third walking leg; 4, fourth walking leg.

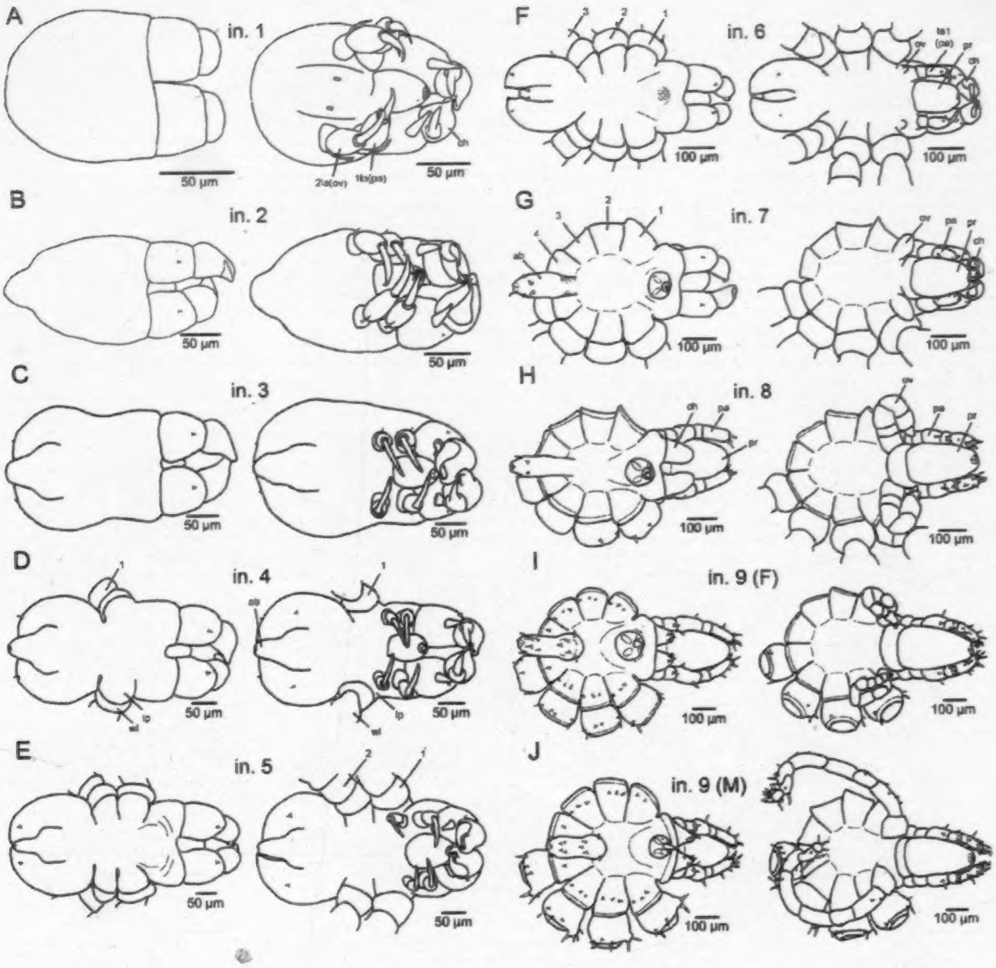
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Figure 3-2. Development of trunk and anterior appendages in *T. bealensis*: A. First instar; B. Second Instar; C. Third Instar; D. Fourth instar; E. Fifth instar; F. Sixth instar; G. Seventh instar; H. Eighth instar; I. Adult female (ninth instar); J. Adult male (ninth instar). Drawings for each instar consist of a dorsal and ventral view of the trunk and anterior appendages. ab, abdomen; ch, chelifore; lp, lateral process; ov, oviger; pa, palp; pr, proboscis; wl, walking leg; 1la(pa), first larval appendage; 2la(ov), second larval appendage; 1, first walking leg; 2, second walking leg; 3, third walking leg; 4, fourth walking leg.

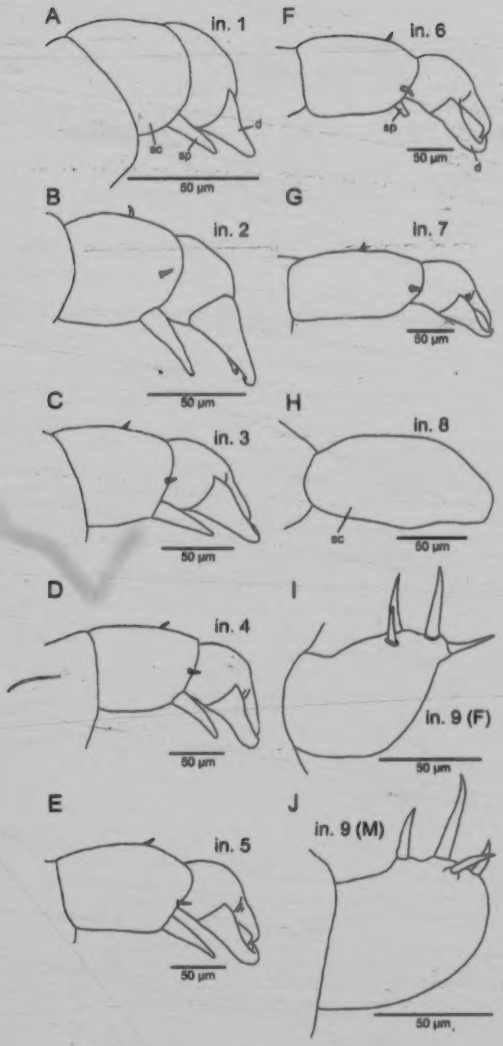
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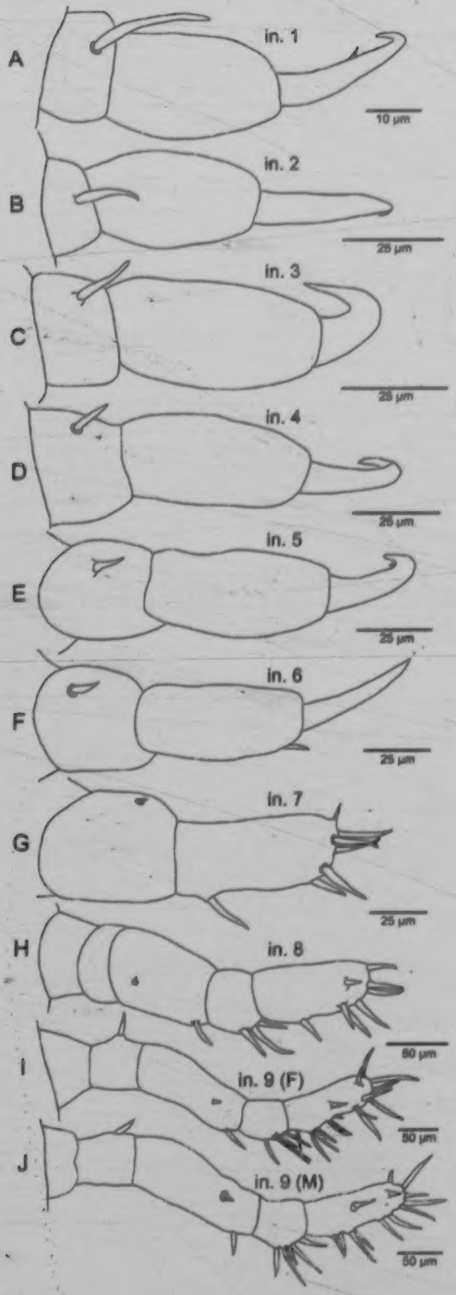
Figure 3-3. Development of the Chelifore in *T. bealensis*: A. First instar; B. Second instar; C. Third instar; D. Fourth instar; E. Fifth instar; F. Sixth instar; G. Seventh instar; H. Eighth instar; I. Adult female (ninth instar); J. Adult male (ninth instar). All drawings are of the right chelifore, lateral view. d, dactyl; sc, scape; sp, spine.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50 µm

Figure 3-4. Development of the Palp in *T. bealensis*: A. First instar; B. Second instar; C. Third instar; D. Fourth instar; E. Fifth instar; F. Sixth instar; G. Seventh instar; H. Eighth instar; I. Adult female (ninth instar); J. Adult male (ninth instar). All drawings are of the right palp, lateral view.

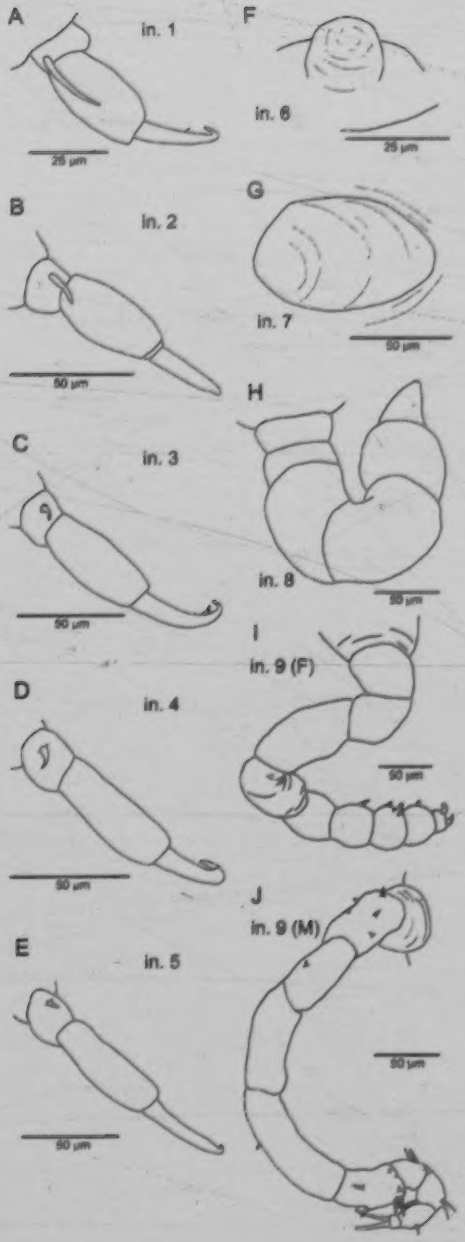
A
in. 1
25 µm
F
in. 6
25 µm
B
in. 2
50 µm
G
in. 7
50 µm
H
in. 8
50 µm
C
in. 3
50 µm
I
in. 9 (F)
50 µm
D
in. 4
50 µm
J
in. 9 (M)
E
in. 5
50 µm
50 µm

Figure 3-5. Development of the Oviger in *T. bealensis*: A. First instar; B. Second instar; C. Third instar; D. Fourth instar; E. Fifth instar; F. Sixth instar; G. Seventh instar; H. Eighth instar; I. Adult female (ninth instar); J. Adult male (ninth instar). All drawings are of the right oviger, lateral view.

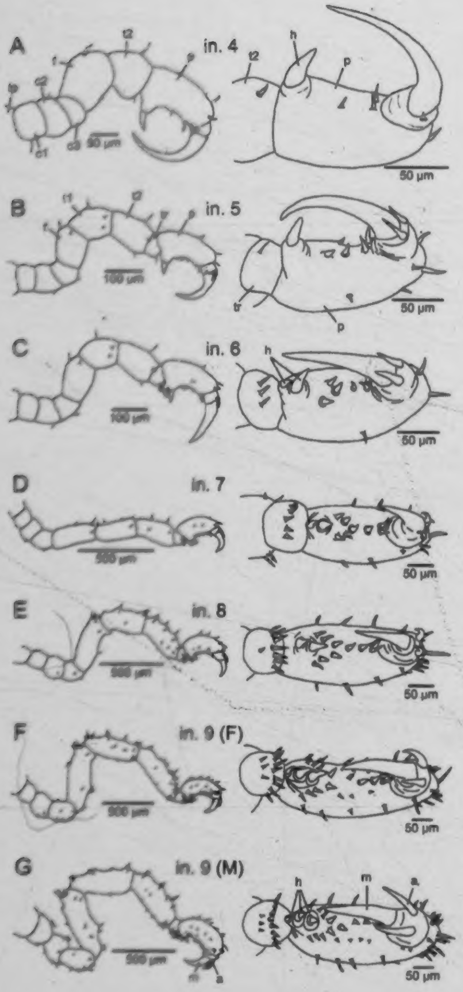
A
in. 4
t2
f
lp
c2
c1
c3
50 µm
h
t2
p
50 µm
B
in. 5
t1
t2
f
tr
p
100 µm
tr
p
50 µm
C
in. 6
100 µm
h
50 µm
D
in. 7
500 µm
50 µm
E
in. 8
500 µm
50 µm
F
in. 9 (F)
500 µm
50 µm
G
in. 9 (M)
500 µm
m
a
h
m
a
50 µm

Figure 3-6. Development of the First Walking Leg in *T. bealensis*: A. Fourth instar; B. Fifth instar; C. Sixth instar; D. Seventh instar; E. Eighth instar; F. Adult female (ninth instar); G. Adult male (ninth instar). Drawings of the first walking leg for each instar consist of the right entire leg (lateral view) and the right terminal segments (ventral view). a, auxillary claw; c1, coxae one; c2, coxae two; f, femur; h, heel spine; lp, lateral process; m, main claw; p, propodus; tr, tarsus; t1, tibia one; t2, tibia two.

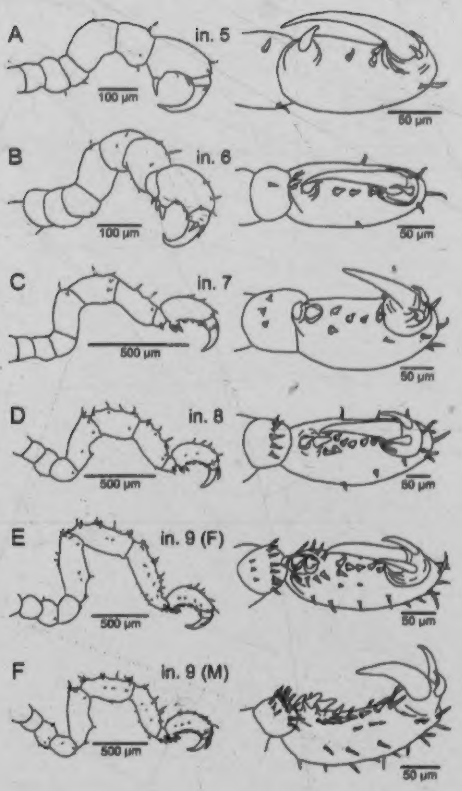
A
in. 5
100 µm
50 µm
B
in. 6
100 µm
50 µm
C
in. 7
500 µm
50 µm
D
in. 8
500 µm
50 µm
E
in. 9 (F)
500 µm
50 µm
F
in. 9 (M)
500 µm
50 µm

Figure 3-7. Development of the Second Walking Leg in *T. bealensis*: A. Fifth instar; B. Sixth instar; C. Seventh instar; D. Eighth instar; E. Adult female (ninth instar); F. Adult male (ninth instar). Drawings of the second walking leg for each instar consist of the right entire leg (lateral view) and the right terminal segments (ventral view).

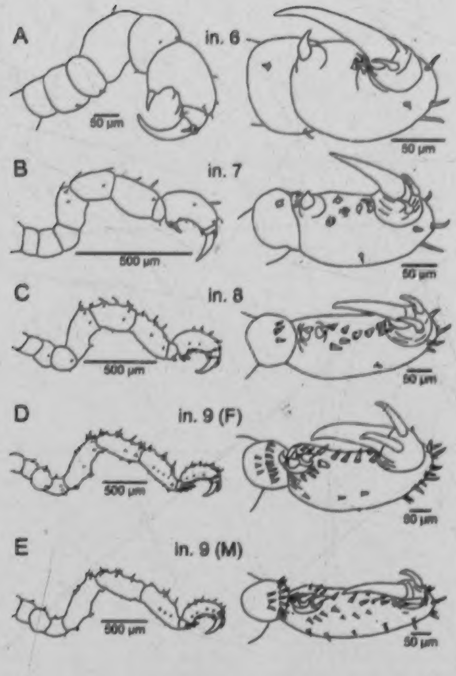
A
in. 6
50 µm
50 µm
B
in. 7
500 µm
50 µm
C
in. 8
500 µm
50 µm
D
in. 9 (F)
500 µm
50 µm
E
in. 9 (M)
500 µm
50 µm

Figure 3-8. Development of the Third Walking Leg in *T. bealensis*: A. Sixth instar; B. Seventh instar; C. Eighth instar; D. Adult female (ninth instar); E. Adult male (ninth instar). Drawings of the third walking leg for each instar consist of the right entire leg (lateral view) and the right terminal segments (ventral view).

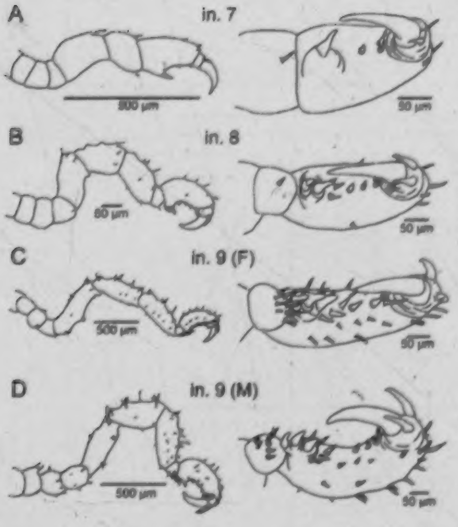
A
in. 7
500 µm
50 µm
B
in. 8
50 µm
50 µm
C
in. 9 (F)
500 µm
50 µm
D
in. 9 (M)
500 µm
50 µm

Figure 3-9. Development of the Fourth Walking Leg in *T. bealensis*: A. Seventh instar; B. Eighth instar; C. Adult female (ninth instar); D. Adult male (ninth instar). Drawings of the fourth walking leg for each instar consist of the right entire leg (lateral view) and the right terminal segments (ventral view).

CHAPTER 4*

Phylogenetic relations of sea spiders (Arthropoda: Pycnogonida) inferred from partial sequences of 18S and 28S ribosomal DNA.

4.1 Introduction

In the study of phylogenetic relationships of Arthropods, the Pycnogonida or sea spiders may offer some very important clues. Although their placement remains unresolved, pycnogonids occupy one of two possible prominent positions. First, they are typically assigned to the subphylum Chelicerata, as the sister group to all other chelicerates, or Euchelicerata, which includes the Merostomata and the Arachnida (Giribet and Ribera 1998; Wheeler and Hayashi 1998; Shultz and Regier 2000; Regier and Shultz 2001). Alternatively pycnogonids are not placed in the Chelicerata at all but are placed more basal as the sister group to all other arthropods (Giribet, Edgecombe and Wheeler 2001). Regardless of whether pycnogonids are the basal chelicerate, or basal arthropod, a better understanding of this group is needed in understanding the evolutionary history of the arthropods (Regier and Shultz 1997; Fortey and Thomas 1998).

Pycnogonids are a unique group of exclusively marine invertebrates found throughout the worlds oceans at all depths (Arnaud and Bamber 1987). As they are rarely found in high densities, are often cryptic and have little economic significance, they receive little more than trivial coverage in general Zoology texts and current research (Arnaud and Bamber 1987; Ruppert and Barnes 1994; Arango 2003). Their body size ranges from 2 mm up to 700 mm in leg span. While most are epibenthic, some are interstitial, bathypelagic, commensal and even parasitic. (Child and Harbison 1986; Arnaud and Bamber 1987; Russell and Hedgpeth 1990). Several characteristics make them distinct from other arthropods, including the lack of a respiratory or excretory system, presence of ovigerous legs, and — unique among invertebrates — they possess exclusive paternal care (King 1973; Ridley 1978; Arnaud and Bamber 1987).

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* This chapter will be submitted for publication as a paper co-authored with Dr. Eric Lovely and Dr. Felix Sperling.

In addition to our poor understanding of pycnogonid biology, phylogenetic relations among pycnogonids have troubled biologists for years. Traditionally, pycnogonid classification has been based on the morphology of head appendages (chelifores, palps, and ovigers), and nine families are recognized: Ammotheidae, Austrodecidae Callipallenidae, Colossendeidae, Endeidae, Nymphonidae, Phoxichilidiidae, Pycnogonidae, and Rhynchothoraxidae (Arnaud and Bamber 1987; Hedgpeth 1947; Hedgpeth 1954; Munilla 1999). In recent years, various phylogenetic studies have examined the validity of these nine families using morphological data (Arango 2002; Bain 1992; Lovely 1999). Unfortunately, perhaps because they used different numbers of taxa and characters, all three obtained conflicting results regarding monophyly of all families except Nymphonidae and Ammotheidae. Nymphonidae was always found to be monophyletic. In contrast, Ammotheidae was always found to be para- or polyphyletic. However, in all three studies, trees lacked much statistical support, likely due to the small number of morphological characters in relation to the number of taxa examined. For all three, the number of taxa was equal to or greater than the number of characters included. This provides an inadequate dataset upon which to infer phylogenetic relationships using parsimony (Mitchell *et al* 2000; Felsenstein 1985; Sanderson 1989). The simple morphology of the pycnogonids makes acquiring an adequate number of morphological characters extremely difficult, and continues to be a limitation in resolving their relationships. In addition, their poor representation in the fossil record limits morphological datasets to extant taxa.

Arango (2003) recognized the limited power of morphological data for this group, so used molecular data as an alternative for examining pycnogonid phylogenetic relationships. Using partial sequences of 18S and 28S rDNA from 11 species, she tested the validity of classifications suggested by morphological data. Her analyses yielded three major findings. First, Nymphonidae and Callipallenidae grouped together along with Colossendeidae. Second, Austrodecidae was the basal family, and third the Ammotheidae was polyphyletic. Molecular support for monophyly of Nymphonidae and the polyphyly of Ammotheidae agree with earlier findings of the three morphological phylogenies.

Molecular phylogenetic analyses of pycnogonids are typically limited by the number of species available. Arango (2003) examined 11 species from six families to provide the first molecular phylogenetic reconstruction of pycnogonid relationships. However, many species were not sequenced for both 18S and 28S rDNA (D4-D7 regions), leaving very little overlap between the different genes. Here, we present the second molecular phylogenetic study of the higher level pycnogonid relationships, using a large fragment of 18S rDNA and the D3 region of 28S rDNA. The D3 region of 28S rDNA has been more commonly used for arthropods and thus allows the use of outgroup taxa, which could not be done by Arango (2003) due to use of the D4-D7 region. In addition, we included all sequenced 18S and D3 28S rDNA data currently available from the literature and GenBank, increasing the number of pycnogonid taxa to 36 species representing eight of the nine pycnogonid families. This larger dataset should allow for a more accurate assessment of the relations among pycnogonid families.

4.2 Materials and Methods

4.2.1 Taxon Sampling

Complete 18S rDNA and partial 28S rDNA (D3 region) sequences were obtained for eight of the nine families of pycnogonids, by direct sequencing of specimens and from literature sources (Table 4-1). Specimens directly sequenced in the present study were preserved in 95-99% ethanol and collected from various sites worldwide, either personally or by collaborators (Table 4-2). Vouchers of sequenced specimens will be deposited at the California Academy of Sciences (San Francisco, California, USA, 94118-4599). Pycnogonid sequences obtained from literature sources, as well as outgroup taxa sequences, were obtained through GenBank (accession numbers in Table 4-1). Outgroup taxa were chosen to be as similar as possible to the outgroup taxa used by Arango (2003), and represent the same higher groupings (Table 4-1). However, the same species as Arango (2003) could not be used for all outgroups because both 18S and 28S sequences were required for outgroup taxa. Outgroup taxa selected by Arango (2003) and ourselves were based on the results of Giribet and Ribera (2000), which examined basal phylogenetic relationships of the Arthropoda. While GenBank sequences of the

28S fragment examine are available for both species of Acari included in this study, they were not included in the analysis simply due to an oversight during alignment. This was not fixed due to time constraints.

4.2.2 Molecular Sequencing

Sequencing was done by two different techniques. E.C. Lovely (1999) sequenced twelve specimens (Table 4-1 – footnote a) for the D3 region of the 28S rDNA, while J.M. Gillespie sequenced 18S rDNA and the 28S rDNA D3 fragment for all additional specimens.

DNA Isolation. For J.M. Gillespie (JMG), half of a specimen or a piece of tissue from the leg was air dried, and genomic DNA was then extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). For E.C. Lovely (ECL), DNA was extracted according to Litvaitis *et al.* (1994), using phenol-chloroform.

DNA Amplification. The 18S rDNA was PCR-amplified in four overlapping fragments of about 500-600 bp each, using primer pairs 3F-5R2, 4F-6R2, 5F2-7R, and 6F2-8R, respectively. Primers 3F, 4R, 7R, and 8R were originally described by Giribet *et al.* (1996, 1999), while primers 5F2, 5R2, 6F2, and 6R2 were modified specifically for pycnogonids from the original primers (Table 4-3). The 28S rDNA D3 fragment was PCR-amplified using primers 28Sa and 28Sb (Whiting *et al.*, 1997), also referred to as D3A and D3B primers (Litvaitis *et al.*, 1996) (Table 4-3).

Amplification of contaminant DNA is a concern in all analyses using PCR. To control for contamination during DNA amplification, sterile techniques were used, such as using only sterilized pipette tips, tubes and solutions. PCR was done under a laminar flow hood to reduce the chance of air-borne contaminants. After being sequenced, all amplified products were confirmed to be pycnogonid sequences using a BLAST search. In addition, for 18S rDNA, of overlapping fragments for each specimen were compared to check for discrepancies between fragments.

PCR amplifications for 18S rDNA and 28S rDNA (JMG) were done either in T Gradient or T Personal Thermocycler (Whatman Biometra, Göttingen, Germany) and carried out in a 50 μl volume reaction, with 34.50 μl RNAase-free water, 5.0 μl Taq DNA Polymerase 10X Buffer (Promega), 4.0 μl $MgCl_2$ (25mM; Promega), 1.0 μl dNTPs (6.25 μM; Roche Diagnostics), 2 μl of each primer (5 μM), and 0.5 μl of *Taq* 9 Polymerase (5.0 U); M. Pickard, University of Alberta). The PCR amplification program (JMG) consisted of a 5 min denaturation step at 95°C followed by 35 cycles at 95°C for 45s, 49°C for 1 min, 72°C for 1 min, and a final step at 72°C for 7 min. PCR products were visualized on a 1.5% agarose gel (Gibco BRL), with a DNA molecular weight standard. Occasionally, gel extractions (Qiagen QIAquick Gel Extraction Kit) were performed on PCR products with faint bands, and re-amplified using the gel extract as the template. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen).

PCR amplifications for 28S rDNA (ECL) were done in a Perkin-Elmer 2400. PCR amplification (ECL) consisted of a 2 min denaturation step at 96°C followed by 35 cycles at 94°C for 30s, 45°C for 60s, and 72°C for 120s. Amplified products were electrophoresed on 1% SeaKem agarose gel with a DNA molecular weight standard. The product was excised from the agarose and purified by centrifugation using a Costar Spin-X column (Corning Inc.). Alternatively, amplified DNA was electrophoresed on a 1% SeaPlaque agarose gel. The correct band was cut from the SeaPlaque, melted at 65°C, cooled to 37°C, and the agarose digested using 1.5μl agarose overnight at 37°C.

DNA sequencing. 18S rDNA PCR-purified samples (JMG) were cycle sequenced in both forward and reverse directions using the Amersham Bioscience DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Buckinghamshire, England) The sequencing reaction was carried out in a 15 μl volume reaction: 9 μl RNAase free water, 4 μl of ET Terminator Sequencing Kit, 1 μl of purified PCR product, and 1 μl of primer. The cycling-sequencing program consisted of an initial step at 93°C for 30s, 28 sequencing cycles (95°C for 20 s, 45°C for 15 s, 60°C for 1 min), and a final extension at 60°C for 30 s. ET-labeled PCR products were purified by filtration through Sephadex-packed columns and vacuum-dried. The dried ET-labeled product was re-suspended in formamide and sequenced on an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) at the University of Alberta.

28S rDNA PCR-purified samples (JMG) were cycle sequenced in both forward and reverse directions using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Sequencing reaction was carried out in a 10 μl volume reaction: 3 μl 2.5X reaction buffer, 1 μl of BigDye Reaction Mix, 1-2 μl of purified PCR product, 0.5 μl primer, and 3.5-4.5 μl RNAase free water. The cycling-sequencing program consisted of an initial step at 96°C for 1 min, and 25 sequencing cycles (96°C for 10 s, 50°C for 5 s, 60°C for 4 min). BigDye-labeled PCR products were purified by filtration through Sephadex-packed columns and vacuum-dried. The dried BigDye-labeled product was re-suspended in formamide and sequenced on an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) at the University of Alberta.

28S rDNA PCR-purified (ECL) were cycle sequenced in both forward and reverse directions using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Each product was then sequenced on an ABI Prism 373A automated DNA sequencer (Applied Biosystems, Foster City, CA) at the University of New Hampshire sequencing facility.

Sequence editing. Chromatograms obtained from the automated sequencer were read and contigs made using Sequencher 4.1.2 (Gene Codes Corporation 2000). Sequencher was also used to combine overlapping 18S rDNA sequences. The external primers 3F and 8R (18S rDNA) and 28Sa and 28Sb (28S rDNA) were excluded from the fragments. DNA sequences were aligned using Clustal X (Thompson *et al.* 1997) using the default alignment parameters. Alignments were then manually aligned by eye using MacClade 4.0 (Maddison and Maddison, 2000).

4.2.3 Phylogenetic Analysis

Both genes were analyzed individually and in combination by maximum parsimony (MP) and maximum likelihood (ML) methods. Incongruence between the two datasets was tested using the bootstrap overlap test (Page 1996), bootstrap confidence intervals test (Rodrigo *et al.* 1993), and the randomization test (Rodrigo *et al.*, 1993). These were chosen over the incongruence length difference test (ILD) due to computational and time constraints, and because the ILD test may not be sensitive enough to detect congruence in many cases(Darlu and Lecointre 2002; Barker and Lutzoni 2002). MP methods were compared for incongruence using all three tests.

Phylogenetic analysis of each gene and the combined data set under the maximum parsimony model was performed using heuristic searches with the following parameters: 100 random addition replicates, stepwise addition, and tree bisection-reconnection (TBR). Branch support was calculated using bootstrap values, using 1000 bootstrap replicates and the fast stepwise-addition search. Maximum likelihood analyses were performed using heuristic searches with the following parameters: 100 random addition replicates, stepwise addition, and tree bisection-reconnection (TBR). ML model parameter settings were identified as optimal for each dataset by Modeltest 3.06 (Posada and Crandall 1998). Modeltest obtains a starting tree using Neighbor-joining methods, estimates model parameters based on the starting tree and then calculates the likelihood score for each model. Various models were then compared using hierarchical likelihood ratio tests (hLRTs) to determine the optimal model and parameters for each dataset (Posada and Crandall 2001). Model descriptions can be found in the manual for Modeltest 3.06 (Posada and Crandall 1998).

4.3 Results

4.3.1 18S ribosomal DNA

A total of 1987 nucleotide sites were analyzed in the 18S ribosomal DNA gene. Thirty-eight taxa were examined, including eight outgroup taxa and 30 pycnogonid species representing eight of the nine recognized families. With outgroup taxa included, of the 1987 sites, 548 sites (27.6%) were variable and 333 sites (16.8%) were parsimony informative (Table 4-4). When outgroup taxa were excluded and only pycnogonid taxa included, 315 sites (15.9%) were variable and 149 sites (7.5%) were parsimony informative (Table 4-4). The average base frequencies for 18S rDNA were: A = 24.96%, C = 22.96%, G = 27.80 %, T = 24.28%, with no significant difference in base frequencies among taxa ($X^2 = 26.89$, df = 111, $P = 1.0$). Four hyper-variable regions were seen in the aligned sequence in positions 194-228, 270-310, 780-833, 1485-1537, and 1870-1905 of the 18S rDNA alignment. Numbering based on alignment of sequences used in this study. Uncorrected sequence pairwise divergence (p-distance) among pycnogonids ranged from 0% to 7.0% (mean = 2.4%) and among the outgroup taxa, it ranged from 3.7% to 12.3% (mean = 8.1%). Mean sequence pairwise divergence among the outgroup taxa and the pycnogonids was 8.5%, ranging from 1.7% to 15.1%. The four lowest pairwise divergences (<2%) were between the pycnogonid species *Anoplodactylus portus* and each of two outgroup taxa (*Limulus polyphemus* (Xiphosura) and *Liphistius bicoloripes (*Araneae), and also between another pycnogonid *Anoplodactylus lentus* and the same two outgroup taxa. All of these are GenBank sequences. This suggests the two *Anoplodactylus* sequences may be in error.

Maximum parsimony analysis yielded 1580 shortest trees (Fig. 4-1; L = 1218; CI = 0.64; RI = 0.66). Monophyly of the Pycnogonida was strongly supported by 88% bootstrap support. The Pycnogonida grouped more closely with the myriapod taxa than any of the chelicerate outgroup taxa, and this grouping had 81% bootstrap support. The chelicerate outgroup taxa did not form a monophyletic clade, but were paraphyletic with the Myriapoda and pycnogonids nested within. All pycnogonid families with more than one species sampled were found to be polyphyletic except Nymphonidae, which was paraphyletic. Species of both the Callipallenidae and the Colossendeidae nested inside the Nymphonidae at the top of the tree, but each family also had a species at the basal part of the pycnogonid clade. For example, *Pigrogromitus timsanus* (Callipallenidae) was found to be the basal pycnogonid. *Endeis* (Endeidae) was found to be the sister taxa to the majority of the Ammotheidae.

Maximum likelihood analysis was conducted using the optimal model (TrNef + I + G) preferred under the hLRTs. The ML analysis used a heuristic search with the MP majority rule consensus topology as the starting tree. Model parameters were those determined for the optimal model preferred under the hLRTs (Table 4-4) using Modeltest 3.06 (Posada and Crandall, 1998). 10 equally likely trees were obtained from the heuristic search (Fig. 4-2; -Ln L = 8892.58701). All trees showed the pycnogonids to be monophyletic and to group more closely with the Myriapoda taxa than with any of the chelicerate taxa, as seen in the MP analysis of 18S rDNA. However, the remaining chelicerates formed a monophyletic clade that was found in all trees. All pycnogonid families with more than one species sampled were again found to be polyphyletic except Nymphonidae, which remained paraphyletic, and Pycnogonidae (*Pycnogonum*), which was found to be monophyletic. In this analysis, the Nymphonidae + Callipallenidae + Colossendeidae clade was found near the base of the tree, and *Endeis* (Endeidae) grouped with one of the *Anoplodactylus* species as the sister group to the majority of the Ammotheidae.

4.3.2 28S ribosomal DNA

A total of 390 sites were analyzed for the 28S ribosomal DNA gene. Thirty-five taxa were included in the analysis, including six outgroup taxa and 29 pycnogonid species representing eight of the nine families. With outrgroup taxa included, of the 390 sites, 176 (45.1%) were variable and 123 (31.5%) were parsimony informative (Table 4-4). When outgroup taxa were excluded and only pycnogonid taxa included, 116 sites (29.7%) were variable and 87 (22.3%) were parsimony informative (Table 4-4). The average base frequencies for 28S rDNA were: A = 21.95%, C = 26.86%, G = 32.59 %, T = 18.60%, with no significant difference in base frequencies among taxa ($X^2 = 21.84$, df = 108, $P = 1.0$). One hyper-variable region was seen in the aligned sequence, and was found in position 137-167 of the 28S rDNA alignment. Uncorrected sequence pairwise divergence (p-distance) among pycnogonids ranged from 0% to 17.5% (mean = 9.9%) and among outgroup taxa, it ranged from 9.0% to 21.0% (mean = 15.5%). Mean sequence pairwise divergence between the outgroup taxa and the pycnogonids was 21.0%, ranging from 16.7% to 29.0%.

The MP analysis yielded 281 shortest trees (Fig. 4-3; L = 568; CI = 0.51; RI = 0.67) differing among the pycnogonid and outgroup taxa. Monophyly of the Pycnogonida was strongly supported (100% bootstrap support). The Pycnogonida grouped more closely with the remaining chelicerate taxa than with the myriapod outgroup taxa, and this association was well supported by 85% bootstrap support. The chelicerate outgroup taxa formed a monophyletic clade, while the Myriapoda were paraphyletic. All pycnogonids families with more than one species sampled were found to be polyphyletic except Nymphonidae, Endeidae and Pycnogonidae. Nymphonidae was paraphyletic, while Endeidae and Pycnogonidae were both monophyletic. Species of both the Callipallenidae and the Colossendeidae nested inside the Nymphonidae at the top of the tree, except *Pigrogromitus timsanus* (Callipallenidae), which was found to be the sister group to the majority of the Phoxichilidiidae. *Endeis* (Endeidae) was found to be the sister taxon to *Pigrogromitus* and the Phoxichilidiidae (except *Anoplodactylus portus*). The Pycnogonidae *(Pycnogonum)* was the basal pycnogonid family.

Maximum Likelihood analysis was done on the optimal model (TrNef + G) preferred under the hLRTs. The ML analysis used a heuristic search with the MP majority rule consensus topology as the starting tree. Model parameters were those determined for the optimal model preferred under the hLRTs (Table 4-4) using Modeltest 3.06 (Posada and Crandall, 1998). 6873 best trees were obtained from the heuristic search (Fig. 4-4; -Ln L = 3056.79112), with differences occurring throughout the entire tree. Monophyly of the pycnogonids was only found in 68% of the best trees, with the pycnogonids grouping more closely with the clade consisting of the Myriapoda and Acari taxa than with any of the other chelicerate taxa. All pycnogonid families with more than one species sampled were found to be polyphyletic except Nymphonidae, Endeidae and Pycnogonidae. Nymphonidae was found to be paraphyletic, while Endeidae and Pycnogonidae were found to be monophyletic. The Nymphonidae + Callipallenidae + Colossendeidae clade was only supported in 59% of the best trees, and lacked *Pigrogromitus* (Callipallenidae). *Endeis* (Endeidae) was found to group as the sister taxon to the majority of the Phoxichilidiidae + *Pigrogromitus. Ammothea hilgendorfi* (Ammotheidae) and *Anoplodactylus portus* (Phoxichilidiidae) were placed as the basal pycnogonid taxa.

4.3.3 18S and 28S ribosomal DNA Combined

The tree topology of the 18S and 28S analyses differed in both the outgroup and pycnogonid taxa. The bootstrap overlap test and the bootstrap confidence interval test comparing MP methods of 18S and 28S rDNA indicated that the two datasets were not completely homogeneous. The randomization test indicated that the two datasets were not heterogeneous on both the 18S and 28S null distributions (0.97 and 0.93, respectively). Thus the two datasets are not completely incongruent and could be combined for a total evidence analysis.

The combined dataset consisted of 2377 sites, 724 (30.5%) were variable and 456 (19.2%) were parsimony informative when outgroup taxa were included (Table 4-4). When outgroup taxa were excluded and only pycnogonid taxa included, 431 sites (18.1%) were variable and 236 sites (9.9%) were parsimony informative (Table 4-4). A total of 44 taxa were included in the analysis, consisting of eight outgroup taxa and 34 pycnogonid species representing eight of the nine families. Uncorrected sequence pairwise divergence (*p*-distance) within the pycnogonids ranged from 0% to 17.5% (mean 9.1%) and within the outgroup taxa, it ranged from 3.3% to 12.4% (mean = 9.0%). Mean sequence pairwise divergence between the outgroup taxa and the pycnogonids was 11.4%, ranging from 2.8% to 30.2%.

The MP analysis yielded 1310 shortest trees (Fig. 4-5; L = 1809; CI = 0.64; RI = 0.66) that differed in relations among both pycnogonid and outgroup taxa. Monophyly of the Pycnogonida was found in all of the shortest trees and was strongly supported by 93% bootstrap support. The Pycnogonida grouped more closely with myriapod taxa than any of the chelicerate outgroup taxa, and was well supported by 78% bootstrap support. The chelicerate outgroup taxa did not form a monophyletic clade, but were paraphyletic with the Myriapoda and pycnogonids nested within. All pycnogonid families with more than one species sampled were found to be polyphyletic except Nymphonidae, Endeidae and Pycnogonidae. Nymphonidae was found to be paraphyletic, while Endeidae and Pycnogonidae were monophyletic. Species of both the Callipallenidae and the Colossendeidae nested inside the Nymphonidae near the top of the tree, but each family also had a species near the basal part of the pycnogonid clade. *Pigrogromitus timsanus* (Callipallenidae) was found to be the basal pycnogonid. *Endeis* (Endeidae) was found to be nested within the clade containing the majority of the Ammotheidae.

Maximum Likelihood analysis was conducted using the optimal model (TrNef + I + G) preferred under the hLRTs. The ML analysis used a heuristic search with the MP majority rule consensus topology as the starting tree. Model parameters were those determined for the optimal model preferred under the hLRTs (Table 4-4) using Modeltest 3.06 (Posada and Crandall, 1998). A single optimal tree was obtained from the heuristic search (Fig 4-6; -Ln L = 12154.77437). The pycnogonids were found to be monophyletic and to group more closely with the Myriapoda than with any of the chelicerate taxa, as seen in the MP analysis. All pycnogonid families with more than one species sampled were again found to be polyphyletic except Nymphonidae, Endeidae and Pycnogonidae. Again, Nymphonidae was paraphyletic, while Endeidae and Pycnogonidae were monophyletic. Both the Callipallenidae and the Colossendeidae nested inside the Nymphonidae, but each family also had a species elsewhere in the pycnogonid clade. *Endeis* (Endeidae) and the Phoxichilidiidae were nested with the Ammotheidae. *Pigrogromitus* (Callipallenidae) was the basal pycnogonid.

4.4 Discussion

4.4.1 Outgroup Relationships

Representatives of chelicerates and myriapods were used as outgroup taxa to root the pycnogonids and partially to determine whether pycnogonids should be included in the Chelicerata. The monophyly of pycnogonids was well supported in both datasets and for both optimality criteria. The Myriapoda always formed a monophyletic group as the sister group to the pycnogonids (Figs. 4-1, 4-2, 4-4, 4-5, 4-6), or as the sister group to the chelicerates when the pycnogonids were used to root the tree (Fig. 4-3). The relationships within the chelicerates was consistent among datasets and optimality criteria, except for the 18S rDNA dataset analyzed using maximum parsimony (Fig. 4-1). The mites (Acari; *Ixodes* and *Opilioacarus*) and the scorpion (*Belisarius*) typically were found to be the most derived chelicerates, except in the 18S rDNA dataset with MP, where the opiliones grouped with the mites at the tip of the tree (Fig. 4-1). Separation of the pycnogonids from the chelicerates suggests they should be considered a distinct subphylum. However, this project is not aimed at examining pycnogonid placement within the arthropods and a more thorough analysis including more arthropod taxa is needed before such a relationship can be inferred with confidence.

4.4.2 Basal Pycnogonid Family

Pigrogromitus timsanus (Callipallenidae) appeared as the basal pycnogonid in the 18S analysis (Figs. 4-1, 4-2) and the combined analysis of the two datasets (Figs. 4-5, 4-6). However, the 28S dataset placed *Pigrogromitus* within the Endeidae + Phoxichilidiidae clade, while MP analysis placed the Pycnogonidae basally (Fig. 4-3) and the ML analysis placed *Ammothea hilgendorfi* (Ammotheidae) basally (Fig. 4-4). Nymphonidae (Munilla and de Haro, 1981; Hedgpeth, 1947) or Ammotheidae (Stock, 1994; Munilla, 1999) are typically considered to be the basal pycnogonid taxa due to their similarity to the other chelicerates in terms of number of appendages in the adults (Arango, 2003). Although Callipallenidae is thought to be closely related to the Nymphonidae, as suggested in all three analyses, *Pigrogromitus* was never found to be closely related to the Nymphonidae or the other callipallenid taxa. These results suggest that the status of *Pigrogromitus* should be more closely examined to determine if it truly should be included in the Callipallenidae or should have its own familial status. The ML analysis of the 28S dataset identified *Ammothea hilgendorfi* (Ammotheidae) as the basal taxon (Fig. 4-4), supporting the more traditional view of pycnogonid relationships. However, *A. hilgendorfi* was very unstable in its positions in all analyses, and was one of the ammotheid species that caused the family to be polyphyletic.

Regardless of what species was placed as the basal taxon, bootstrap support for these basal placements was weak, indicating that the basal portion of the pycnogonid phylogeny was highly unstable and could not be resolved confidently by these data. Arango (2003) suggested Austrodecidae might be the basal pycnogonid group, even though it has never been proposed as an early form of sea spider. In both the 18S dataset (Figs. 4-1, 4-2) and the combined datasets (Figs. 4-5, 4-6), Austrodecidae was never found near the base of the pycnogonid topology, and thus the possibility of the Austrodecidae as the basal pycnogonid is not supported here.

4.4.3 Monophyly and Placement of the Colossendeidae

Four species of the Colossendeidae were included in this study, all but *Colossendeis* sp. #2 (GenBank) had sequences for both 18S and 28 rDNA. None of the datasets or analyses performed found Colossendeidae to be monophyletic. Only the *Colossendeis* species obtained from GenBank (*Colossendeis #1 & #2)* consistently grouped somewhere in the Nymphonidae + Callipalleneidae clade, and their placement varied from being the sister group to being species within either family. The other two *Colossendeis* species (*C. robustus* & *C. megalonyx*) showed no stable affinities and their placement fluctuated dramatically, ranging from sister group to the Nymphonidae + Callipalleneidae clade to being closely related to some of the Ammotheid genera. The position of the Colossendeidae with the Nymphonidae + Callipalleneidae clade agrees with the molecular analysis by Arango (2003). The position of the Colossendeidae with ammotheid genera agrees with the morphological results by Arango (2002), and with the traditional classifications (Hedgpeth, 1947; Arango, 2003). The polyphyly of the Colossendeidae was not seen by Arango (2003) because she examined only a single species.

4.4.4 Is Endeis a Phoxichilidiid?

The genus *Endeis* is generally accepted to be part of a distinct family, the monotypic Endeidae (Hedgpeth, 1947; King 1973; Child 1992), but Stock (1965) suggested that it be included as part of the family Phoxichilidiidae. Arango's (2002) morphological data grouped *Endeis* with Phoxichilidiidae based on a few morphological synapomorphies. However, when Arango (2003) examined molecular data, there was no support for *Endeis* grouping with any other pycnogonid family. The placement of *Endeis* in this project was highly unstable and was never strongly supported (see figs 4-3, 4-5). In the 18S dataset (Figs. 4-1, 4-2) and combined datasets (Figs. 4-5, 4-6), *Endeis* was found either to be nested within part of the Ammotheidae along with or without the single phoxichilidiid *Anoplodactylus tenuicorpus* or as the sister group to some ammotheids. In the 28S dataset, the monophyletic *Endeis* clade was found to be the sister group to the majority of the Phoxichilidiidae (Figs. 4-3, 4-4). Regardless of the position of *Endeis* in any dataset, it was never nested within the Phoxichilidiidae, and therefore there was no support for Stock's suggestion that it be included in the Phoxichilidiidae. However, the

28S dataset suggested that the family Endeidae may be the sister group to the Phoxichilidiidae, which would agree with Munilla (1999).

4.4.5 Status of the Ammotheidae

The family Ammotheidae is the largest pycnogonid family with 31 genera and over 350 species. The morphological characters that define this family are extremely diverse and new species are placed in this family more as a grouping of convenience than based on phylogenetic relationships. Every investigation on pycnogonid phylogenetic relationships using phylogenetics has found the group to be either para- or polyphyletic (Bain, 1992; Lovely; 1999; Arango, 2002; Arango, 2003). A major revision of the family is clearly needed (Fry & Hedgpeth, 1969), as an acceptable resolution has yet to be proposed. Arango (2003) suggested that the problem is the limited taxon sampling of the ammotheid genera and that more extensive sampling is needed before relationships within this lineage will be resolved. Arango (2003) used three genera with four species to represent the Ammotheidae in her molecular analysis, while six genera with 13 species were used in this study. Although the number of genera and species included has more than doubled in this study, it only represents one-fifth of the genera and only 4% of all ammotheid species. While Arango (2003) found moderate support for the monophyly of the Ammotheidae using molecular data, previous work using morphological data suggested paraphyly (Arango, 2002). The results of this study are mixed, with some ammotheids consistently grouping together as a monophyletic clade, and others varying in their position within the pycnogonids. So this study also strongly suggests the Ammotheidae are polyphyletic. While several other taxa change position in the topology depending on the dataset and the optimality criteria, three particular species of the genus *Ammothea* were the most unstable: *A. gracialis, A. hilgendorfi,* and *A. spinosa*. This suggests that the genus *Ammothea* was the source of conflict with the ammotheids examined, but one species of the genus (*A. marginatus*) was always found within the clade that contained the majority of the species.

To further complicate the uncertain status of the ammotheids, an examination of the monophyly of the four genera that contained multiple species showed that only the genus *Tanystylum* was monophyletic. The genus *Achelia* is generally thought to be the sister group to the genus *Tanystylum* and this relationship is seen in the present study. In all datasets and for both optimality criteria, two of the three species of *Achelia* group with *Tanystylum*. This is supported by bootstrap numbers ranging from 55% up to 90%. The lack of monophyly among the ammotheid genera examined suggested that it is not only familial status that needs to be reexamined, but also the monophyly of currently accepted genera. Since only a small fraction of species were included in this study, more extensive sampling, as initially suggested by Arango (2003), is needed before the true status of the family Ammotheidae and its genera can be resolved.

4.4.6 Summary

This study builds upon the work of Arango (2003) to reconstruct pycnogonid relationships using molecular data by adding additional taxa and additional sequence for 18S and 28S rDNA. The present study re-confirms that traditional classifications for pycnogonids persist strictly for convenience and do not represent true evolutionary relationships. However, phylogenetic studies have, as of yet, been unable to suggest a clear alternative and traditional classification should continue to be used as a matter of convenience and stability. Although a revision of pycnogonid classification is necessary, it cannot be resolved by the taxa and sequences presented here. Additional taxa need to be included in analyses to provide a greater presentation of the diversity found within the pycnogonids. As well, additional genes such as elongation factor -1α (EF-1α) and elongation factor 2, need to be sequenced to provide additional data upon which proposed phylogenetic relationships can be examined. Because of their possible basal placement in the arthropods, an understanding of their phylogenetic relationships is crucial to our understanding of the origin of the Pycnogonida, and potentially to the early history of arthropods.

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Table 4-1. Taxon sampling of pycnogonids and outgroup taxa used in this study

Higher Group	Taxon	18S rDNA	28S rDNA (D3)
Pycnogonida			
Ammotheidae	*Achelia chelata*	X	X
	Achelia gracilipes	X	X
	Achelia echinata	AF005438[b]	AF005459[b]
	Ammothea gracialis	X	X[a]
	Ammothea hilgendorfi	X	X
	Ammothea marginatus	X	X
	Ammothea spinosa	----	X[a]
	Ammothella tuberculata	X	X
	Ammothella sp. CPA-2002	AF448552[c]	----
	Ascorhynchus ramipes	AF448555[c]	----
	Cilunculus sp.	----	X[a]
	Tanystylum californicum	X	X
	Tanystylum bealensis	X	X
Austrodecidae	*Austrodecus childi*	AF448554[c]	----
Callipallenidae	*Pigrogromitus timsanus*	X	X
	Callipallene sp. GG-2000	AF005439[b]	AF005460[b]
Colossendeidae	*Colossendeis megalonyx*	----	X[a]
	Colossendeis robustus	X	X[a]
	Colossendeis sp.#1	AF005440[b]	AF005461[b]
	Colossendeis sp.#2	AF062946[d]	----
Endeidae	*Endeis laevis*	AF005441[b]	AF005462[b]
	Endeis spinosa	----	X[a]
Nymphonidae	*Nymphon australe*	----	X[a]
	Nymphon grossipes	X	X[a]
	Nymphon micronesium	AF448556[c]	----
	Nymphon pixellae	X	X
	Nymphon spinosissimum	X	X
	Nymphon sp.	U88338[e]	----
Phoxichilidiidae	*Anoplodactylus lentus*	AF062945[d]	AF062797[d]
	Anoplodactylus portus	AF062944[d]	AF062771[d]
	Anoplodactylus tenuicorpus	AF448565[c]	----
	Phoxichilidium femoratum	X	X
	Phoxichildium quadridentatum	X	X
	Phoxichildium tubulariae	----	X[a]
Pycnogonidae	*Pycnogonum littorale*	X	X[a]
	Pycnogonum stearnsi	X	X
Outgroup Taxa			
Subclass Merostomata			
Order Xiphosura	*Limulus polyphemus*	U91490[b]	U91492[b]
Subclass Arachnida			
Order Acari	*Ixodes uriae*	AF115369[f]	----
	Opilioacarus texanus	AF115375[f]	----
Order Scorpiones	*Belisarius xambeui*	AF005442[b]	AF124954[b]
Order Araneae	*Liphistius bicoloripes*	AF007104[b]	AF124960[b]
Order Opilione	*Parasiro coffaiti*	U36999[b]	U91495[b]
Subphylum Myriapoda			
Class Chilopoda	*Scutigera coleoptrata*	AF000772[b]	AF000779[b]
Class Diplopoda	*Cylindroiulus punctatus*	AF005448[b]	AF005463[b]

Sequences reported by: [a] - Lovely (1999); [b] - Giribet & Ribera (2000); [c] - Arango (2003); [d] - Wheeler & Hayashi (1998); [e] - Aleshin *et al.* (1998); [f] - Klompen, J.S.H., Black, W.C. IV, Keirans, J.E. and Norris, D.E. (unpublished); X - specimens sequenced in this study.

Table 4-2. Localities of pycnogonids sequenced by the authors

	Collecting Location	Collector
Pycnogonida		
AMMOTHEIDAE		
Achelia chelata	Duxbury, California, USA	J.M. Gillespie
Achelia gracilipes	Barkley Sound, BC, Canada	J.M. Gillespie
Ammothea gracialis	Cape Evans, Antarctica	E.C. Lovely
Ammothea hilgendorfi	Kirby Point, California, USA	J.M. Gillespie
Ammothea marginatus	Barkley Sound, BC, Canada	J.M. Gillespie
Ammothea spinosa	Granite Harbor, Antarctica	E.C. Lovely
Ammothella tuberculata	Barkley Sound, BC, Canada	J.M. Gillespie
Cilunculus sp.	Arrival Heights, Antarctica	E.C. Lovely
Tanystylum californicum	Dillon Beach, California, USA	J.M. Gillespie
Tanystylum bealensis	Barkley Sound, BC, Canada	J.M. Gillespie
CALLIPALLENIDAE		
Pigrogromitus timsanus	Long Key, Florida, USA	T. McGovern / S. Boyce
COLOSSENDIDAE		
Colossendeis megalonyx	Arrival Heights, Antarctica	E.C. Lovely
Colossendeis robustus	Cape Armitage, Antarctica	E.C. Lovely
ENDEIDAE		
Endeis spinosa	Arrabida, Portugal	E.C. Lovely
NYMPHONIDAE		
Nymphon australe	Granite Harbor, Antarctica	E.C. Lovely
Nymphon grossipes	Portsmouth, New Hampshire, USA	E.C. Lovely
Nymphon pixellae	Barkley Sound, BC, Canada	J.M. Gillespie
Nymphon spinosissimum	Carnwallis Island, Nunavut, Canada	K. Schnabel
PHOXICHILIDIIDAE		
Phoxichilidium femoratum	Barkley Sound, BC, Canada	J.M. Gillespie
Phoxichildium quadridentatum	Doran Beach, California, USA	J.M. Gillespie
Phoxichildium tubulariae	Portsmouth, New Hampshire, USA	E.C. Lovely
PYCNOGONIDAE		
Pycnogonum littorale	Eastport, Maine, USA	E.C. Lovely
Pycnogonum stearnsi	Dillon Beach, California, USA	J.M. Gillespie

Table 4-3. Primers used in amplification and sequencing

Primer	5'-3'	
18S rDNA		
3F	GTT CGA TTC CGG AGA GGG A	(19bp)
4F	CCA GCA GCC GCG (CG)TA ATT C	(19bp)
5R	CTT GGC AAA TGC TTT CGC	(18bp)
5F2	GCG AAA GCA TT(TC) GCC AAG AA	(20bp)
6R2	ATT CCT TTA AGT TTC AGC T	(19bp)
6F2	AGC TGA AAC TTA AAG GAA T	(19bp)
7R	GCA TCA CAG ACC TGT TAT TGC	(21bp)
8R	ACG GGC GGT GTC TAC	(15bp)
28S rDNA		
28Sa / D3A	GAC CCG TCT TGA AAC ACG GA	(20bp)
28Sb / D3B	TCG GAA GGA ACC AGC TAC TA	(20bp)

Table 4-4. Summary of dataset statistics and model parameters.

	18S rDNA	28S rDNA	18S+28S rDNA Combined
Number of taxa	32 P, 8 O	29 P, 6 O	36 P, 8 O
Characters included	1987	390	2377
Variable (including outgroup)	548 (27.6%)	176 (45.1%)	724 (30.5%)
Parsimony-informative (including outgroup)	333 (16.8%)	123 (31.5%)	456 (19.2%)
Variable (excluding outgroup)	315 (15.9%)	116 (29.7%)	431 (18.1%)
Parsimony-informative (excluding outgroup)	149 (7.5%)	87 (22.3%)	236 (9.9%)
Length MPT	1218	568	1809
Number MPT	1580	281	1310
CI	0.64	0.51	0.64
RC	0.66	0.67	0.65
Model hLRTs	TrNef + I + G	TrNef + G	TrNef + I + G
	$I = 0.6092$; $G = 0.4580$	$G = 0.4580$	$I = 0.5144$; $G = 0.3217$
	Rate Matrix R:	Rate Matrix R:	Rate Matrix R:
	AC 1.00000	AC 1.00000	AC 1.00000
	AG 2.26448	AG 1.79804	AG 2.19924
	AT 1.00000	AT 1.00000	AT 1.00000
	CG 1.00000	CG 1.00000	CG 1.00000
	CT 4.09947	CT 5.11168	CT 4.60941
	GT 1.00000	GT 1.00000	GT 1.00000
Number MLT	10	6873	1
-Ln L	8892.58701	3056.79112	12154.77437

P = number of pycnogonid taxa
O = number of outgroup taxa
CI = consistency index
RC = rescaled consistency index
MPT = most parsimonious trees

TrNef: 1) equal base frequencies
2) transition - transversions rate not equal
3) transition rates not equal
4) transversion rates equal

I = invariable sites parameter
G = gamma distribution

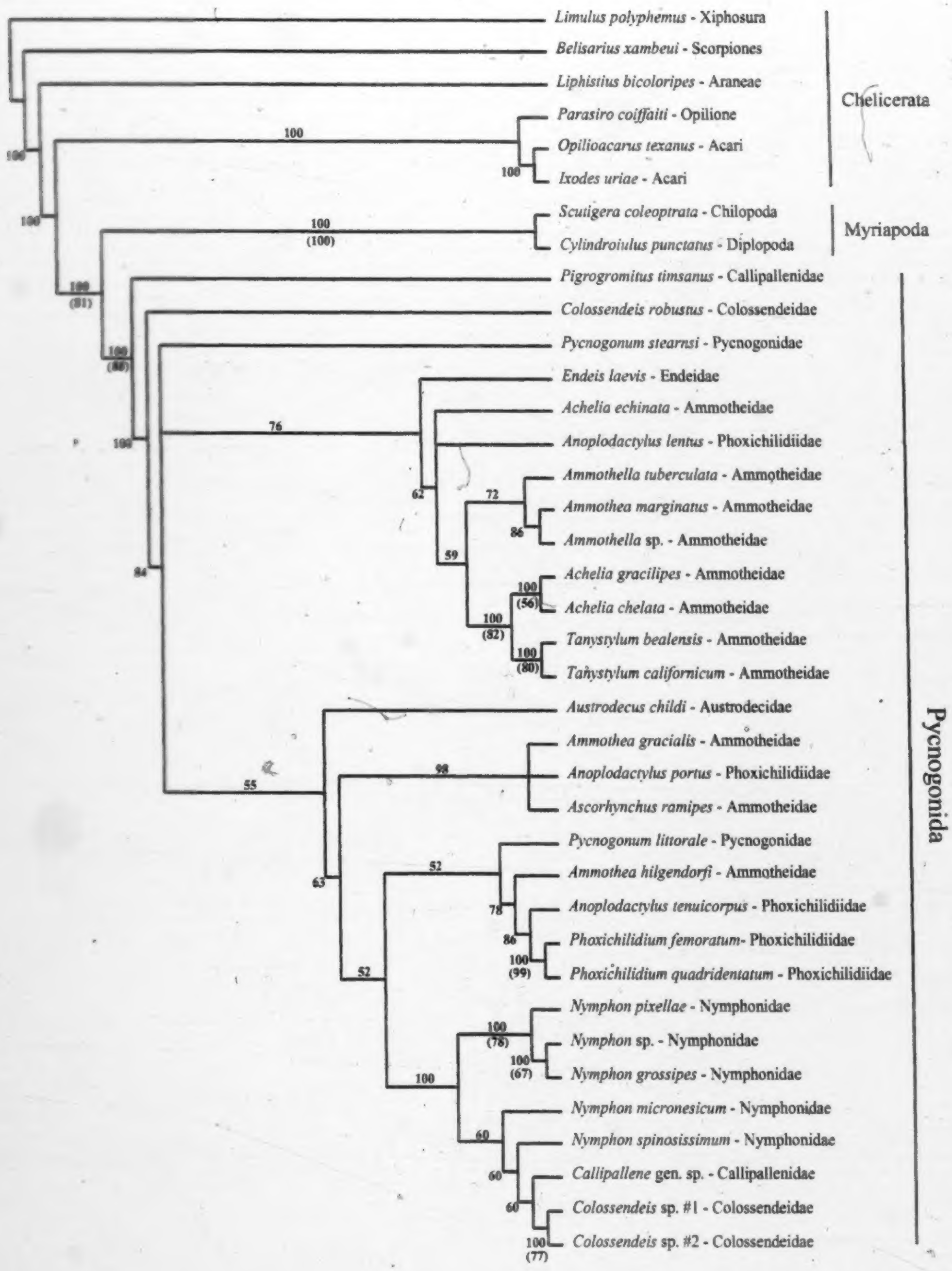
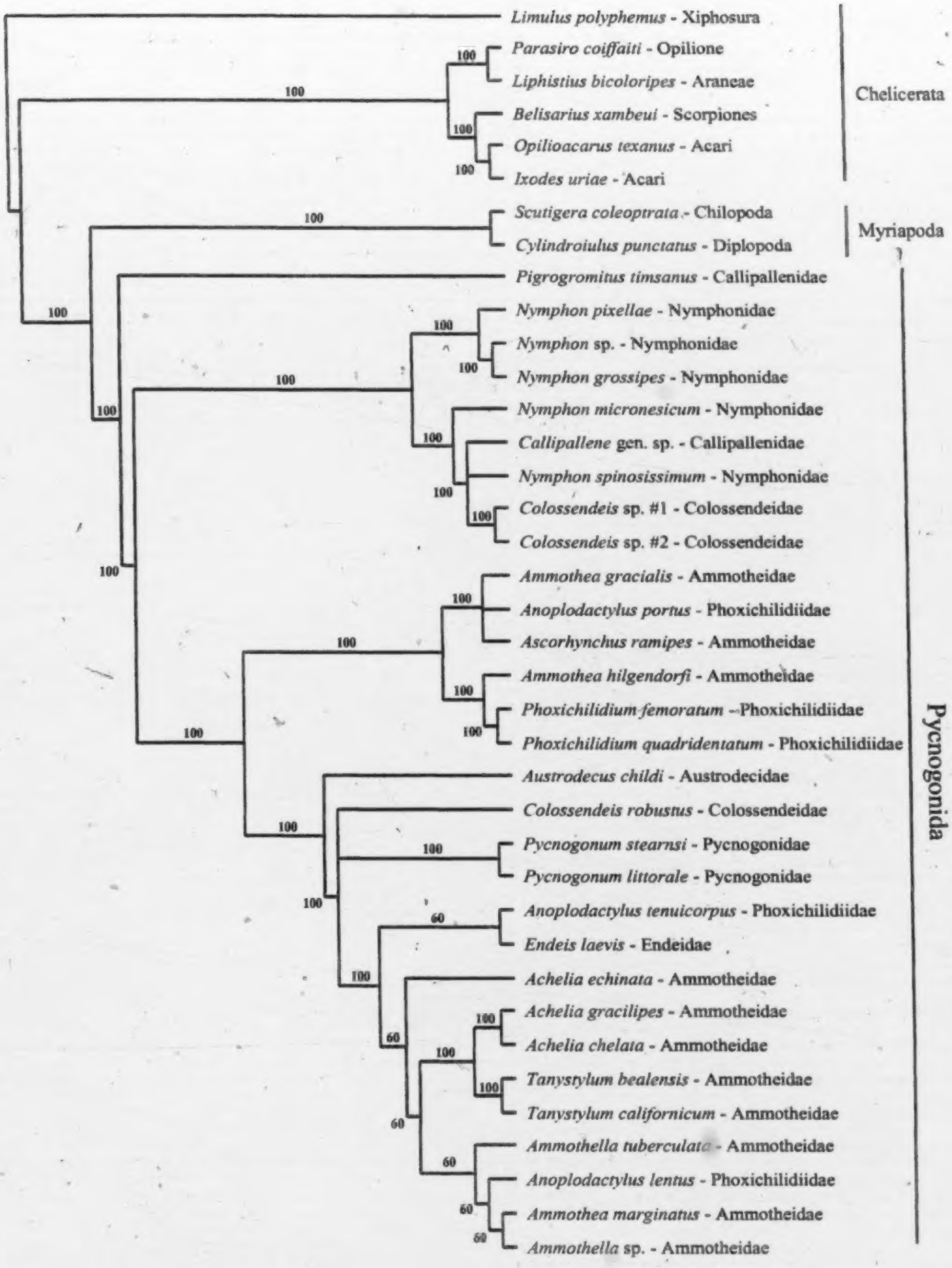

Limulus polyphemus - Xiphosura
Belisarius xambeui - Scorpiones
Liphistius bicoloripes - Araneae
Parasiro coiffaiti - Opilione
Opilioacarus texanus - Acari
Ixodes uriae - Acari
Scutigera coleoptrata - Chilopoda
Cylindroiulus punctatus - Diplopoda
Pigrogromitus timsanus - Callipallenidae
Colossendeis robustus - Colossendeidae
Pycnogonum stearnsi - Pycnogonidae
Endeis laevis - Endeidae
Achelia echinata - Ammotheidae
Anoplodactylus lentus - Phoxichilidiidae
Ammothella tuberculata - Ammotheidae
Ammothea marginatus - Ammotheidae
Ammothella sp. - Ammotheidae
Achelia gracilipes - Ammotheidae
Achelia chelata - Ammotheidae
Tanystylum bealensis - Ammotheidae
Tanystylum californicum - Ammotheidae
Austrodecus childi - Austrodecidae
Ammothea gracialis - Ammotheidae
Anoplodactylus portus - Phoxichilidiidae
Ascorhynchus ramipes - Ammotheidae
Pycnogonum littorale - Pycnogonidae
Ammothea hilgendorfi - Ammotheidae
Anoplodactylus tenuicorpus - Phoxichilidiidae
Phoxichilidium femoratum- Phoxichilidiidae
Phoxichilidium quadridentatum - Phoxichilidiidae
Nymphon pixellae - Nymphonidae
Nymphon sp. - Nymphonidae
Nymphon grossipes - Nymphonidae
Nymphon micronesicum - Nymphonidae
Nymphon spinosissimum - Nymphonidae
Callipallene gen. sp. - Callipallenidae
Colossendeis sp. #1 - Colossendeidae
Colossendeis sp. #2 - Colossendeidae
Chelicerata
Myriapoda
Pycnogonida
100
100
100
100
100
(100)
100
(81)
100
(80)
100
76
62
72
86
59
100
(56)
100
(82)
100
(80)
84
55
98
63
52
78
86
100
(99)
52
100
(78)
100
(67)
100
60
60
60
100
(77)

Figure 4-1. 50% Majority rule phylogenetic tree of Pycnogonida based on 18S rDNA obtained under maximum parsimony (1580 trees, $L = 203$, CI = 0.64, and RC = 0.66). Majority rule consensus values above, bootstrap values below branches in brackets.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Parasiro coiffaiti - Opilione
Liphistius bicoloripes - Araneae
Belisarius xambeui - Scorpiones
Opilioacarus texanus - Acari
Ixodes uriae - Acari
Chelicerata
Scutigera coleoptrata - Chilopoda
Cylindroiulus punctatus - Diplopoda
Myriapoda
Pigrogromitus timsanus - Callipallenidae
Nymphon pixellae - Nymphonidae
Nymphon sp. - Nymphonidae
Nymphon grossipes - Nymphonidae
Nymphon micronesicum - Nymphonidae
Callipallene gen. sp. - Callipallenidae
Nymphon spinosissimum - Nymphonidae
Colossendeis sp. #1 - Colossendeidae
Colossendeis sp. #2 - Colossendeidae
Ammothea gracialis - Ammotheidae
Anoplodactylus portus - Phoxichilidiidae
Ascorhynchus ramipes - Ammotheidae
Ammothea hilgendorfi - Ammotheidae
Phoxichilidium femoratum - Phoxichilidiidae
Phoxichilidium quadridentatum - Phoxichilidiidae
Austrodecus childi - Austrodecidae
Colossendeis robustus - Colossendeidae
Pycnogonum stearnsi - Pycnogonidae
Pycnogonum littorale - Pycnogonidae
Anoplodactylus tenuicorpus - Phoxichilidiidae
Endeis laevis - Endeidae
Achelia echinata - Ammotheidae
Achelia gracilipes - Ammotheidae
Achelia chelata - Ammotheidae
Tanystylum bealensis - Ammotheidae
Tanystylum californicum - Ammotheidae
Ammothella tuberculata - Ammotheidae
Anoplodactylus lentus - Phoxichilidiidae
Ammothea marginatus - Ammotheidae
Ammothella sp. - Ammotheidae
Pycnogonida
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
100
60
100
100
60
100
100
60
60
60
60

Figure 4-2. 50% Majority rule phylogenetic tree of Pycnogonida based on 18S rDNA obtained under maximum likelihood (TrNef + I + G model, 10 trees, -Ln = 8892.59). Majority rule consensus values given above branches.

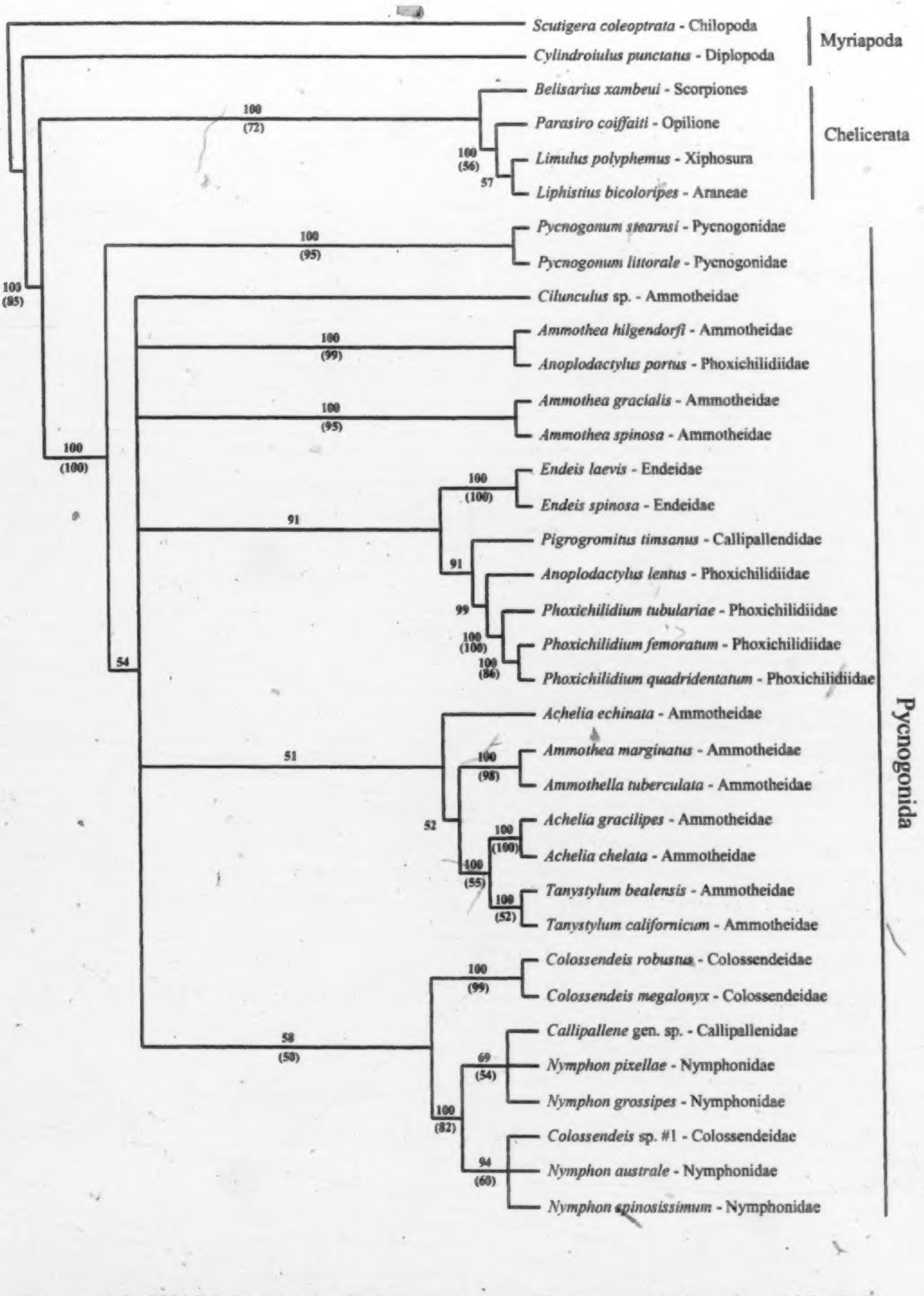
Scutigera coleoptrata - Chilopoda
Cylindroiulus punctatus - Diplopoda
Belisarius xambeui - Scorpiones
Parasiro coiffaiti - Opilione
Limulus polyphemus - Xiphosura
Liphistius bicoloripes - Araneae
Pycnogonum stearnsi - Pycnogonidae
Pycnogonum littorale - Pycnogonidae
Cilunculus sp. - Ammotheidae
Ammothea hilgendorfi - Ammotheidae
Anoplodactylus portus - Phoxichilidiidae
Ammothea gracialis - Ammotheidae
Ammothea spinosa - Ammotheidae
Endeis laevis - Endeidae
Endeis spinosa - Endeidae
Pigrogromitus timsanus - Callipallendidae
Anoplodactylus lentus - Phoxichilidiidae
Phoxichilidium tubulariae - Phoxichilidiidae
Phoxichilidium femoratum - Phoxichilidiidae
Phoxichilidium quadridentatum - Phoxichilidiidae
Achelia echinata - Ammotheidae
Ammothea marginatus - Ammotheidae
Ammothella tuberculata - Ammotheidae
Achelia gracilipes - Ammotheidae
Achelia chelata - Ammotheidae
Tanystylum bealensis - Ammotheidae
Tanystylum californicum - Ammotheidae
Colossendeis robustus - Colossendeidae
Colossendeis megalonyx - Colossendeidae
Callipallene gen. sp. - Callipallenidae
Nymphon pixellae - Nymphonidae
Nymphon grossipes - Nymphonidae
Colossendeis sp. #1 - Colossendeidae
Nymphon australe - Nymphonidae
Nymphon spinosissimum - Nymphonidae
Myriapoda
Chelicerata
Pycnogonida
100 (72)
100 (56)
57
100 (95)
100 (85)
100 (99)
100 (95)
100 (100)
100 (100)
91
91
99
100 (100)
100 (86)
54
100 (98)
51
52
100 (100)
100 (55)
100 (52)
100 (99)
58 (50)
69 (54)
100 (82)
94 (60)

Figure 4-3. 50% Majority rule phylogenetic tree of Pycnogonida based on 28S rDNA obtained under maximum parsimony (281 trees, $L = 568$, CI = 0.51, and RC = 0.67). Majority rule consensus values above, bootstrap values below branches in brackets.

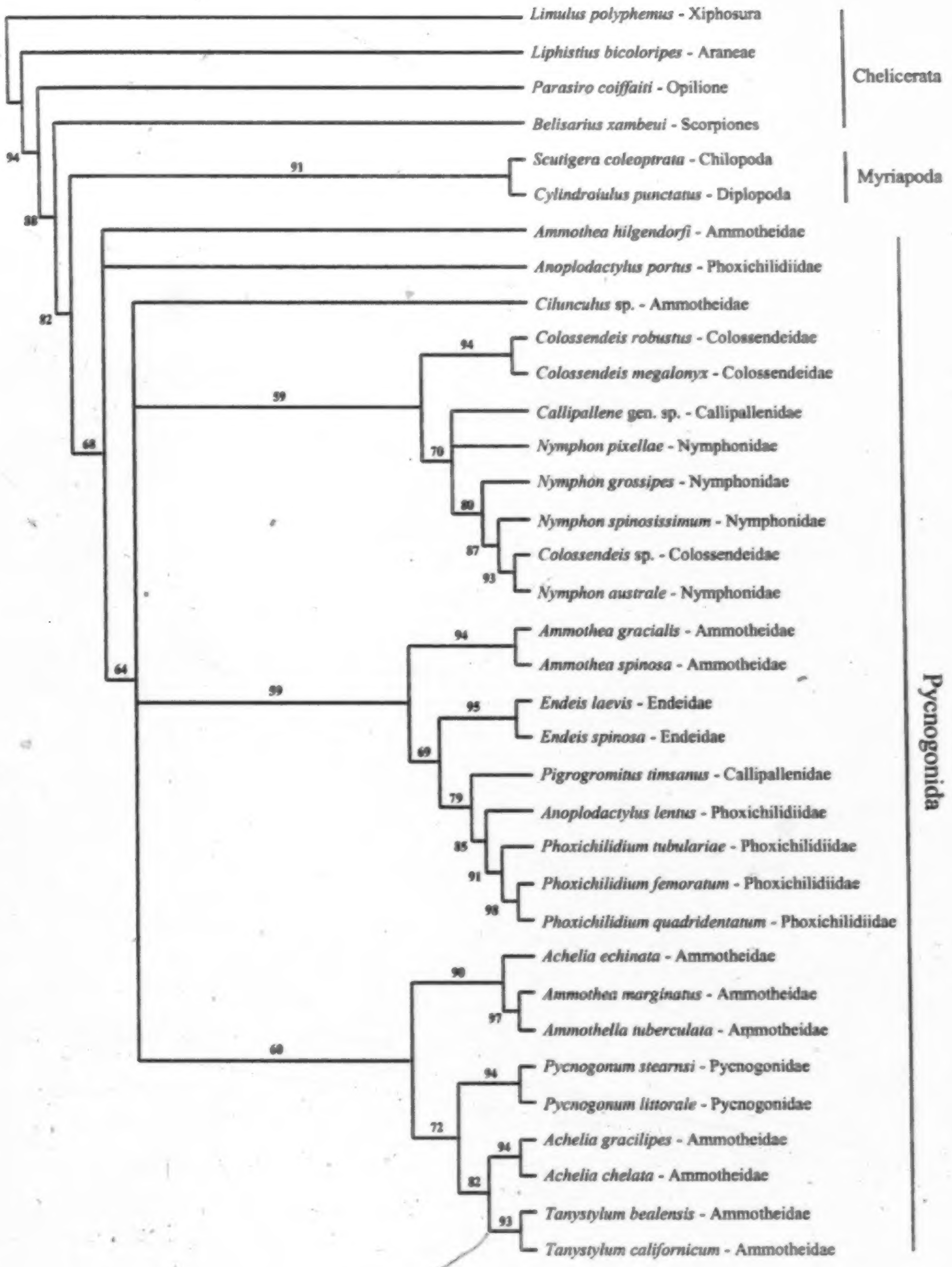
Limulus polyphemus - Xiphosura
Liphistius bicoloripes - Araneae
Parasiro coiffaiti - Opilione
Belisarius xambeui - Scorpiones
Scutigera coleoptrata - Chilopoda
Cylindroiulus punctatus - Diplopoda
Ammothea hilgendorfi - Ammotheidae
Anoplodactylus portus - Phoxichilidiidae
Cilunculus sp. - Ammotheidae
Colossendeis robustus - Colossendeidae
Colossendeis megalonyx - Colossendeidae
Callipallene gen. sp. - Callipallenidae
Nymphon pixellae - Nymphonidae
Nymphon grossipes - Nymphonidae
Nymphon spinosissimum - Nymphonidae
Colossendeis sp. - Colossendeidae
Nymphon australe - Nymphonidae
Ammothea gracialis - Ammotheidae
Ammothea spinosa - Ammotheidae
Endeis laevis - Endeidae
Endeis spinosa - Endeidae
Pigrogromitus timsanus - Callipallenidae
Anoplodactylus lentus - Phoxichilidiidae
Phoxichilidium tubulariae - Phoxichilidiidae
Phoxichilidium femoratum - Phoxichilidiidae
Phoxichilidium quadridentatum - Phoxichilidiidae
Achelia echinata - Ammotheidae
Ammothea marginatus - Ammotheidae
Ammothella tuberculata - Ammotheidae
Pycnogonum stearnsi - Pycnogonidae
Pycnogonum littorale - Pycnogonidae
Achelia gracilipes - Ammotheidae
Achelia chelata - Ammotheidae
Tanystylum bealensis - Ammotheidae
Tanystylum californicum - Ammotheidae
Chelicerata
Myriapoda
Pycnogonida
94
88
82
91
68
64
59
94
70
80
87
93
94
59
95
69
79
85
91
98
90
97
60
94
72
94
82
93

Figure 4-4. 50% Majority rule phylogenetic tree of Pycnogonida based on 28S rDNA obtained under maximum likelihood (TrNef + I model, 6873 trees, -Ln = 3056.79). Majority rule consensus values given above branches.

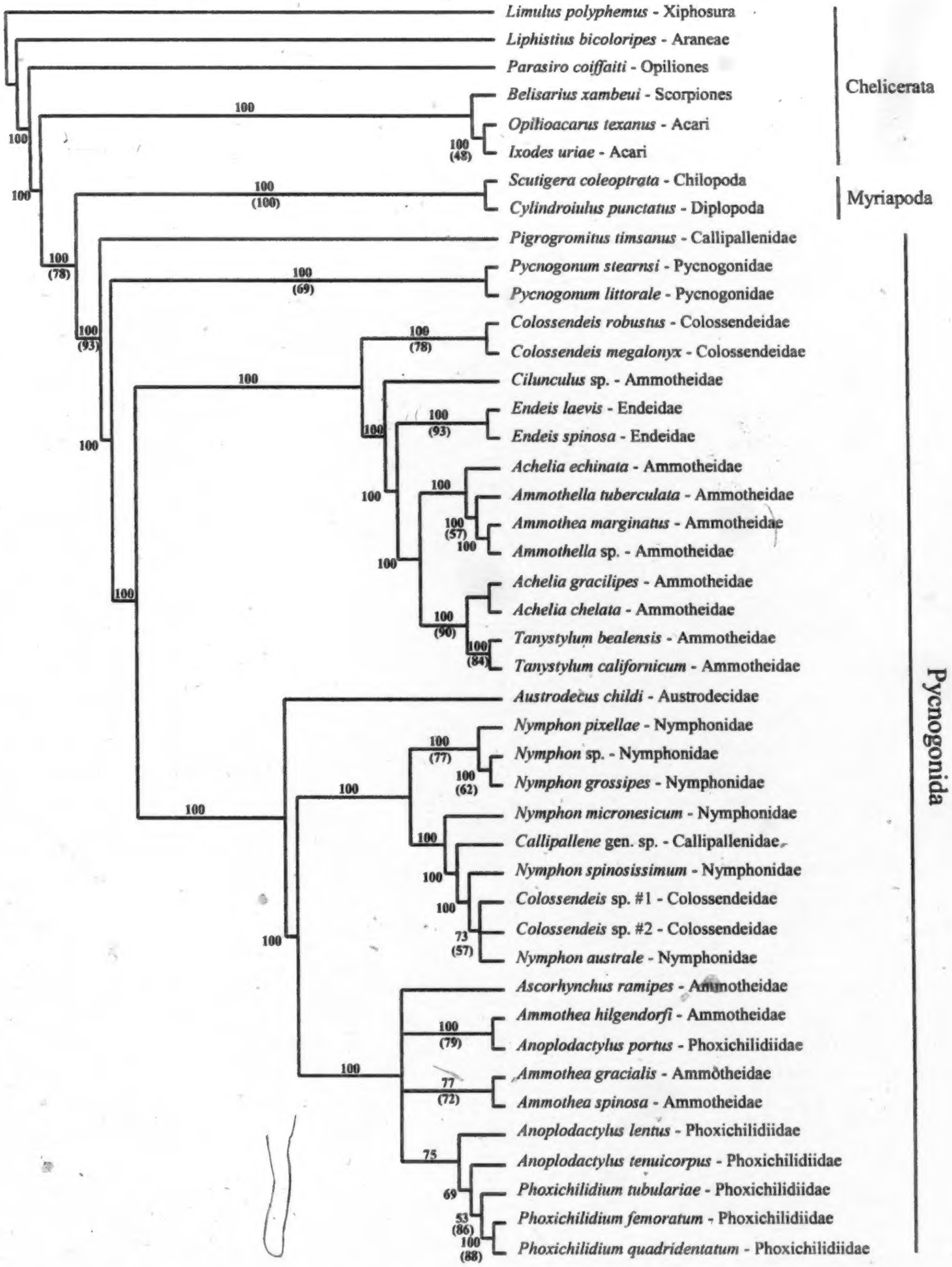
Limulus polyphemus - Xiphosura
Liphistius bicoloripes - Araneae
Parasiro coiffaiti - Opiliones
Belisarius xambeui - Scorpiones
Opilioacarus texanus - Acari
Ixodes uriae - Acari
Chelicerata
Scutigera coleoptrata - Chilopoda
Cylindroiulus punctatus - Diplopoda
Myriapoda
Pigrogromitus timsanus - Callipallenidae
Pycnogonum stearnsi - Pycnogonidae
Pycnogonum littorale - Pycnogonidae
Colossendeis robustus - Colossendeidae
Colossendeis megalonyx - Colossendeidae
Ciluncuius sp. - Ammotheidae
Endeis laevis - Endeidae
Endeis spinosa - Endeidae
Achelia echinata - Ammotheidae
Ammothella tuberculata - Ammotheidae
Ammothea marginatus - Ammotheidae
Ammothella sp. - Ammotheidae
Achelia gracilipes - Ammotheidae
Achelia chelata - Ammotheidae
Tanystylum bealensis - Ammotheidae
Tanystylum californicum - Ammotheidae
Austrodecus childi - Austrodecidae
Nymphon pixellae - Nymphonidae
Nymphon sp. - Nymphonidae
Nymphon grossipes - Nymphonidae
Nymphon micronesicum - Nymphonidae
Callipallene gen. sp. - Callipallenidae
Nymphon spinosissimum - Nymphonidae
Colossendeis sp. #1 - Colossendeidae
Colossendeis sp. #2 - Colossendeidae
Nymphon australe - Nymphonidae
Ascorhynchus ramipes - Ammotheidae
Ammothea hilgendorfi - Ammotheidae
Anoplodactylus portus - Phoxichilidiidae
Ammothea gracialis - Ammotheidae
Ammothea spinosa - Ammotheidae
Anoplodactylus lentus - Phoxichilidiidae
Anoplodactylus tenuicorpus - Phoxichilidiidae
Phoxichilidium tubulariae - Phoxichilidiidae
Phoxichilidium femoratum - Phoxichilidiidae
Phoxichilidium quadridentatum - Phoxichilidiidae
Pycnogonida
100
100
100 (48)
100
100 (100)
100 (78)
100 (69)
100 (93)
100 (78)
100
100
100 (93)
100
100
100 (57)
100
100
100
100 (90)
100 (84)
100
100 (77)
100 (62)
100
100
100
100
73 (57)
100
100
100 (79)
77 (72)
75
69
53 (86)
100 (88)

Figure 4-5. 50% Majority rule phylogenetic tree of Pycnogonida based on combined 18S + 28S rDNA obtained under maximum parsimony (1310 trees, L = 1809, CI = 0.64, and RC = 0.65). Majority rule consensus values above, bootstrap values below branches in brackets.

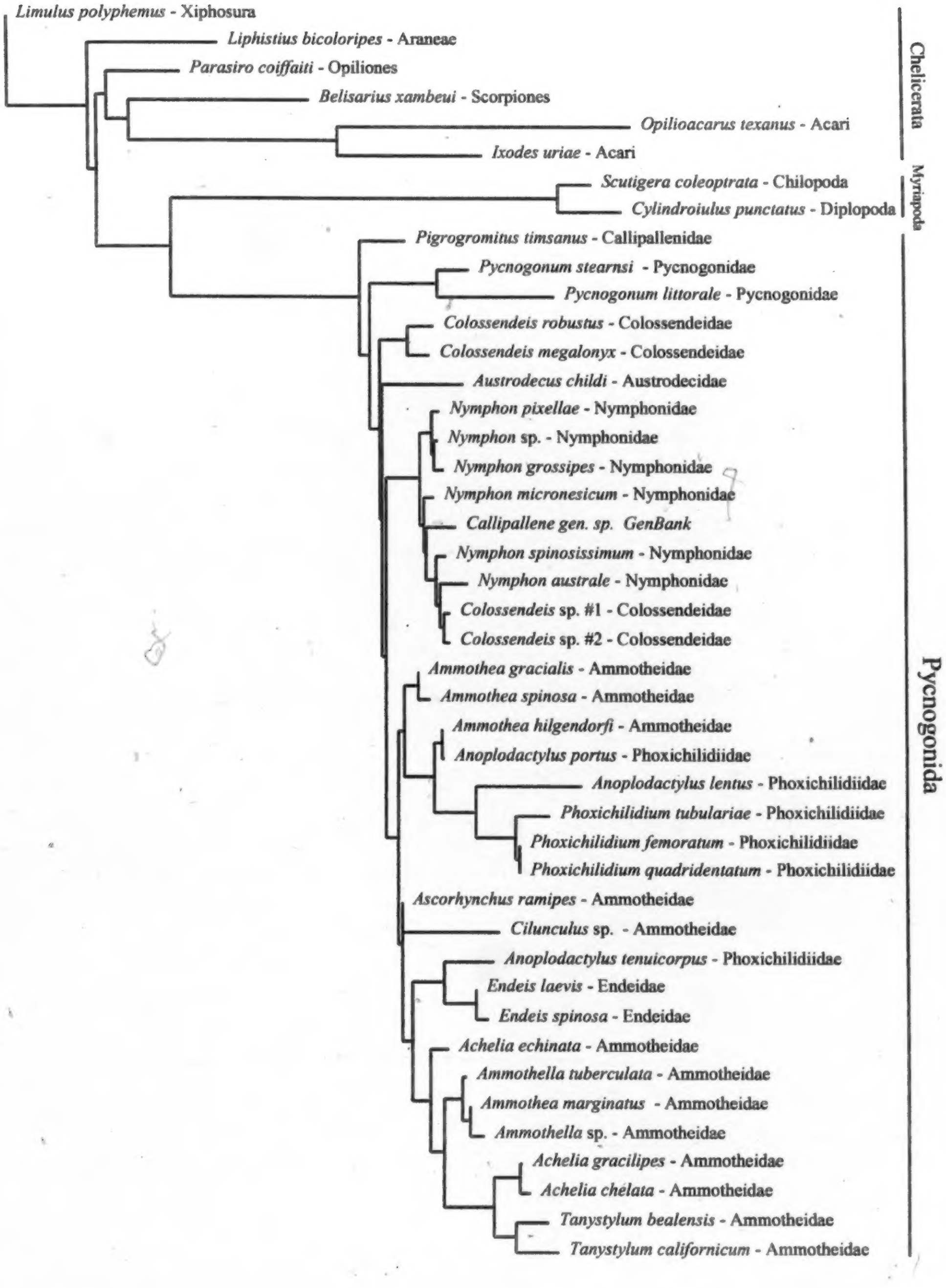
Limulus polyphemus - Xiphosura
Liphistius bicoloripes - Araneae
Parasiro coiffaiti - Opiliones
Belisarius xambeui - Scorpiones
Opilioacarus texanus - Acari
Ixodes uriae - Acari
Scutigera coleoptrata - Chilopoda
Cylindroiulus punctatus - Diplopoda
Pigrogromitus timsanus - Callipallenidae
Pycnogonum stearnsi - Pycnogonidae
Pycnogonum littorale - Pycnogonidae
Colossendeis robustus - Colossendeidae
Colossendeis megalonyx - Colossendeidae
Austrodecus childi - Austrodecidae
Nymphon pixellae - Nymphonidae
Nymphon sp. - Nymphonidae
Nymphon grossipes - Nymphonidae
Nymphon micronesicum - Nymphonidae
Callipallene gen. sp. GenBank
Nymphon spinosissimum - Nymphonidae
Nymphon australe - Nymphonidae
Colossendeis sp. #1 - Colossendeidae
Colossendeis sp. #2 - Colossendeidae
Ammothea gracialis - Ammotheidae
Ammothea spinosa - Ammotheidae
Ammothea hilgendorfi - Ammotheidae
Anoplodactylus portus - Phoxichilidiidae
Anoplodactylus lentus - Phoxichilidiidae
Phoxichilidium tubulariae - Phoxichilidiidae
Phoxichilidium femoratum - Phoxichilidiidae
Phoxichilidium quadridentatum - Phoxichilidiidae
Ascorhynchus ramipes - Ammotheidae
Cilunculus sp. - Ammotheidae
Anoplodactylus tenuicorpus - Phoxichilidiidae
Endeis laevis - Endeidae
Endeis spinosa - Endeidae
Achelia echinata - Ammotheidae
Ammothella tuberculata - Ammotheidae
Ammothea marginatus - Ammotheidae
Ammothella sp. - Ammotheidae
Achelia gracilipes - Ammotheidae
Achelia chelata - Ammotheidae
Tanystylum bealensis - Ammotheidae
Tanystylum californicum - Ammotheidae
Chelicerata
Myriapoda
Pycnogonida

Figure 4-6. Phylogenetic tree of Pycnogonida based on combined18S + 28S rDNA obtained under maximum likelihood (TrNef + I + G model, 1 tree, -Ln = 12154.774).

CHAPTER FIVE

General Discussion

5.1 Overview

The study of sea spiders (Arthropoda: Pycnogonida) is a field of opportunity where topics of research are determined by the material that is available. Because so little is known about them, important new discoveries can be made as opportunities arise (such as the discovery of a new population or new species, etc.). This thesis took advantage of such opportunities to improve our knowledge of the Pycnogonida. Three important aspects of pycnogonid biology were addressed where current knowledge was currently lacking: taxonomy, development and phylogenetic relationships using molecular data.

5.2 Pycnogonid Taxonomy

Current research publications on pycnogonids continue to be dominated by taxonomy, and consist of species descriptions, species revisions, and catalogues of species for various regions throughout the world. The most prevalent taxonomic publication continues to be new species descriptions (Arango 2003), indicative of our general lack of knowledge concerning pycnogonids.

Collections along the Pacific coast of British Columbia over the course of this thesis have revealed that this region is in need of detailed exploration for pycnogonids. Although the pycnogonids of the NE Pacific remain poorly known and require a thorough examination (Child, 1990), the Canadian fauna (British Columbia) is particularly poorly understood. This lack of knowledge can be illustrated by the absence of new species descriptions from the region, where the most recent description was *Nymphon pixellae* (Scott, 1913). During my field research, many different species of pycnogonids were collected, including a new species, *Tanystylum bealensis* (Chapter Two). This species is so abundant along the outer coast that it may be one of the most common pycnogonids in British Columbia, yet it had not been described. A survey of the pycnogonid collection at the Royal British Columbia Museum (RBCM) in Victoria, B.C. also unearthed a new species (*Oropallene* sp.) among the 25 species represented (Appendix A). Many species in American waters (Alaska; Washington to California) are thought to occur in British

Columbia, but remain undocumented. The most comprehensive documented list of the Pacific Canadian fauna dates back to Giltay (1934) where only three species were described. If the known American fauna for the NE Pacific are assumed to extend into Canadian waters, 129 species potentially exist (Appendix A), while currently only 35 species have been reported (Appendix A).

One of the more interesting characters to be discovered from SEM photographs was hollow-tipped spines with bifurcated sides (Chapter 2, Fig. 2-7). These may prove useful as a additional morphological character to distinguish the different pycnogonid groups.

5.3 Pycnogonid Development

To appreciate the significance of a newly discovered species, an understanding of its developmental stages is key. In addition, many museum collections contain a variety of instars adding to confusion in species identification. A lack of developmental knowledge reduces the taxonomic value of such specimens. Therefore, a more complete understanding of post-embryonic development can help identify new species, properly describe existing species, and clarify species synonyms.

This thesis addressed such issues with a complete description of the nine instars of post-embryonic development in the newly described *Tanystylum bealensis*. Although this description was not used in the new species description, several post-embryonic differences were found between *T. bealensis* and two other species. The first interesting aspect of the post-embryonic development was the appearance of the walking legs over several instars, initially six-segmented, and then eight-segmented in the following instar. This pattern is consistent with that found in the closely related *T. orbiculare*, but is markedly different from the more distantly related *Achelia alaskensis,* where walking legs may initially be six- or seven-segmented. Second, *T. bealensis* developed over nine instars, while *A. alaskensis* has ten. By examining the differences between two species and two sister genera, post-embryonic development may be useful for correctly identifying instars down to genus. In addition, by understanding the variation in development across a range of pycnogonid species, we may be able to infer evolutionary trends in development throughout Pycnogonida, which in turn might provide clues about ancestral arthropod developmental patterns.

5.4 Pycnogonid Phylogenetic Relationships

Although new species descriptions and a better understanding of post-embryonic development aids in taxon placement within pycnogonids, a focus on the families at the molecular level may help resolve even more of the phylogenetic problems that plague this group. Previous molecular phylogenies (Arango 2003) suggest that Nymphonidae, Callipallenidae and Colossendeidae form a monophyletic clade. In addition, Arango's analysis suggested that Austrodecidae was the basal family and that Ammotheidae was polyphyletic. However, these relationships were based on analyses of 11 species from six families and many species were not sequenced for both 18S and 28S rDNA. The present study examined the same relationships using a larger fragment of 18S rDNA and used the D3 region of the 28S rDNA on 36 species from 8 families. Several differences from the Arango (2003) phylogeny were found. First, the basal group in the 18S and combined (18S and 28S) analysis was Callipallenidae, while in 28S it was either Pycnogonidae or Ammotheidae. Second, only two species of Colossendeidae consistently grouped together in the Nymphonidae + Callipallenidae clade, while the other two grouped with either the Nymphonidae or Ammotheidae. Although the trees inferred here differ in several ways from those of Arango (2003), the Ammotheidae still remain a polyphyletic group. Overall, the topology presented by this study and that of Arango (2003) revealed that the traditional phylogeny is poorly supported, but these later studies were unable to provide much additional resolution. As a result, the traditional phylogeny should continue to be used so as to provide stability and continuity to the field.

5.5 Future Directions

While many aspects of pycnogonid biology remain to be explored or resolved, future research will depend heavily on successful collection of additional specimens. The Pacific coast of Canada has the potential for harboring many previously described species, as well as the distinct likelihood of additional new species. There is great need for continued exploration, not only along the Canadian coast, but throughout the world.

The primary limitation to improving our understanding of pycnogonids is the ability for individuals to focus on basic taxonomic questions. Natural history is the key to the resolution of the pycnogonids. To begin to understand the pycnogonids, one must spend countless hours scouring dredges and shorelines collecting and observing these organisms. This time investment and accumulation of basic biological knowledge is imperative to the discovery of new species and for any advancement of the field. Only with additional species will we be able to identify the intermediate species linking the families and provide resolution to the pycnogonid classification. Detailed surveys of post-embryonic development may provide characters useful for identifying species, genera or higher ranks and aid in inferring relationships between these groups. Information on habitat preference and prey type may help provide insight into the evolution of the wide range of morphological variation seen within the pycnogonids. Exploration of the mechanisms of reproduction is needed as much remains unknown, for example the undescribed method of reproduction in the Colossendeidae (Arnaud and Bamber 1987). The field of pycnogonid research is endless and will require time, more material and a wide range of collaboration to begin to fill in these and many other holes. While there are many fields of study that can be used to study the pycnogonids, it is our lack of knowledge of their basic biology that is most troubling. Only when we are able to understand the pycnogonids, will we be able to trace their evolution and understand the relevance of pycnogonids to the evolution of the arthropods.

5.6 Literature Cited

Arango, C.P. 2003. Molecular approach to the phylogenetics of sea spiders (Arthropoda: Pycnogonida) using partial sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution.* (in press)

Arnaud F., Bamber, R.N. 1987. The Biology of Pycnogonida. *Advances in Marine Biology.* 24: 1-96.

Child, C.A. 1990. *Nymphon stipulum,* a new pycnogonid species from Southern California. *Beaufortia.* 41: 39-43.

Giltay, L. 1934. Pycnogonida from the coast of British Columbia. *The Canadian Field Naturalist.* 48: 49-50.

Scott, F.M. 1913. On a species of *Nymphon* from the North Pacific. *Annals and Magazine of Natural History, Series 8*. 10:206-208; VII.

APPENDIX A

Checklist of the RBCM Pycnogonid Collection, Catalogue of Potential British Columbia Pycnogonids, Keys to Families and Genera, and notes on range and # species for all pycnogonid genera.

A.1 Introduction

The pycnogonids of the west coast of North America are poorly known, and are in need of reexamination and, in some cases, redescription (Child 1990a). The first major examination of the pycnogonids of the NE Pacific was by Cole (1904) and was the result of the Harriman Alaska Expedition, which sampled from Southern California up to the Pribilof Islands. From Cole (1904) to Giltay (1934), lists of field collections and several new species descriptions were the only contributions to pycnogonid biology and were sporadically reported by Hall (1912; 1913) and Hilton (1915; 1916; 1920). Giltay (1934) provided the first record of pycnogonids in British Columbia waters, while Exline (1936) provided the first account of pycnogonids of nearby Puget Sound, Washington. From 1939 through the early 1950's, the pycnogonid fauna of the NE Pacific was described by J.W. Hedgpeth and W.A. Hilton with an emphasis on the fauna of California (Hedgpeth 1939; 1940; 1941; 1943; 1951; Hilton 1939; 1942a,b,c,d,e,f,g; 1943a,b). The majority of species known today were described during this time. However, species descriptions done by Hilton were typically inadequate, being too short, and lacking sufficient detail and illustrations. It wasn't until the work of C.A. Child (1975; 1996) that Hilton's species were properly redescribed and there was resolution of Hilton's numerous species synonyms. C.A Child (1979; 1987; 1990a,b; 1992; 1994; 1995a; 1996; 1999; 2000) has taken over from work of Hedgpeth and Hilton, providing numerous summaries of the pycnogonid fauna from various regions of the Pacific, as well as adding extensively to the number of species known from this region.

This appendix contains an accumulation of notes made during my master's degree. It is presented here with the hopes that the next graduate student or researcher to take on the challenge of pycnogonid taxonomy of British Columbia or the NE Pacific has a place to start and does not have to struggle as I have through the confusion of the primary literature on pycnogonids, especially for this region of the world. Found within this appendix is a list of the specimens in the collection of the Royal British Columbia

Museum (RBCM; Victoria, BC), as well as a list of potential species to be found in or near the waters of British Columbia, including all synonyms and authorities. I have included a key to the nine families of pycnogonids (based on adults) and for each family have provided information on the diagnostic characteristics, genera and number of species known, a key to the major genera (based on adults), and finally notes on the ranges known for each genus. I have also included a drawing of general pycnogonid morphology to assist with the terminology used in the keys (Figure A-1).

The collection of the RBCM was made available to me through an NSERC Systematics supplement scholarship in association with the Department of Natural History at the RBCM. This collection has been amassed over the past 110 years and the excellent state of preservation of the collection is a credit to the curation it has received. The collection consists of 232 specimens representing 25 species, belonging to 13 genera and 6 families. The majority of the collection is from the waters of British Columbia, but also contains material from the Arctic and Antarctic regions. I would like to express my appreciation to Kelly Sendall, Phil Lambert and Jim Cosgrove, RBCM, for providing me with the opportunity to examine the collection and providing facilities during my visit to the museum.

A.2 Pycnogonids of the Royal British Columbia Museum (RBCM) Collections

- full geographical details are available on RBCM website database or from the collection manager, Invertebrates, Fish and Herpetology.

A.2.1 Family Ammotheidae Dohrn, 1881

Achelia alaskensis (Cole, 1904)
- RBCM 84-00012 (1 female)
- RBCM 990-00896-010 (specimen missing)

Achelia gracilipes (Cole, 1904)
- RBCM 973-00142-002 (1 male, 1 juv)
- RBCM 973-00156-075 (1 juv)
- RBCM 976-00266-002 (1 juv)
- RBCM 976-00466-002 (1 juv)
- RBCM 983-00022-003 (1 male, 1 female, 6 juv)

Ammothella tuberculata Cole, 1904
- RBCM 973-00156-076 (1 juv)
- RBCM 973-00156-077 (3 males)
- RBCM 973-00156-078 (1 male)
- RBCM 973-00159-040 (1 male)

Ascorhynchus japonicum Ives, 1891
  - RBCM 975-00163-001 (sex unknown)

Tanystylum bealensis Gillespie and Bain, 2004
  - RBCM 973-00159-013 (1 male)
  - RBCM 973-00249-002 (9 males, 5 females, 18 juv)
  - RBCM 973-00249-017 (8 males, 7 females, 6 juv)
  - RBCM 976-01081-026 (1 juv)
  - RBCM 002-00001-001 (2 males, 2 females)
  - RBCM 002-00002-001 (1 male, 3 juv)

Tanystylum californicum Hilton, 1939
  - RBCM 975-00771-002 (1 male, 1 female)

Tanystylum grossifemora (Hilton, 1942g)
  - RBCM 974-00568-018 (3 juv)

A.2.2 Family Callipallenidae Hilton, 1942c

Oropallene sp. (NEW SPECIES, undescribed)
  - RBCM 980-00242-010 (1 male)

Pseudopallene circularis (Goodsir, 1842)
  - RBCM 974-00223-059 (1 male)
  - RBCM 976-00033-007 (4 males, 1 female)
  - RBCM 976-00033-008 (17 males, 4 females)

A.2.3 Family Colossendeidae Hoek, 1881

Colossendeis microsetosa Hilton, 1943a
  - RBCM 984-00106-001 (1 male)

Colossendeis megalonyx Hoek, 1881
  - RBCM 975-00735-004 (1 male)

Hedgpethia dofleini (Loman, 1911)
  - RBCM 980-00595-001 (unknown sex)

A.2.4 Family Nymphonidae Wilson, 1878

Boreonymphon compactum Just, 1972
  - RBCM 975-00137-001 (1 male)
  - RBCM 975-00136-001 (1 female)

Nymphon duospinum (Hilton, 1942a)
  - RBCM 974-00384-017 (1 female)

Nymphon grossipes (Fabricius, 1780)
  - RBCM 976-00111-014 (1 male)
  - RBCM 976-00111-016 (1 male, 2 females)
  - RBCM 980-00250-014 (1 male)
  - RBCM 980-00257-025 (1 male)
  - RBCM 985-00469-052 (1 female)
  - RBCM 986-00096-028 (1 male)

Nymphon pixellae Scott, 1913
- RBCM 138-00023 (1 male, 1 female)
- RBCM 140-00025 (1 male)
- RBCM 973-00232-004 (1 male)
- RBCM 975-00072-004 (1 male)
- RBCM 975-00759-010 (1 male)
- RBCM 975-00802-068 (1 male)
- RBCM 975-00802-069 (2 females)
- RBCM 976-00019-001 (1 male, 2 females)
- RBCM 976-00111-015 (2 males)
- RBCM 976-00111-029 (4 females)
- RBCM 976-00115-030 (3 males, 2 females)
- RBCM 976-01023-001 (1 male)
- RBCM 976-01182-004 (4 males, 2 females)
- RBCM 976-01182-005 (1 female)
- RBCM 979-11024-004 (2 males)
- RBCM 981-00215-012 (7 males, 5 females)
- RBCM 983-01629-002 (1 male)
- RBCM 983-01636-029 (1 male)
- RBCM 985-00465-060 (2 females)
- RBCM 985-00469-097 (1 male)
- RBCM 988-00010-033 (1 male)
- RBCM 988-00263-001 (2 males)
- RBCM 988-00263-017 (2 males)
- RBCM 988-00744-001 (1 female)
- RBCM 990-00314-020 (15 males, 3 females)
- RBCM 990-00316-003 (2 females)

Nymphon sluiteri Hoek, 1881
- RBCM 69-0061 (1 male)
- RBCM 975-00072-025 (1 male)
- RBCM 975-00136-003 (2 males)

Nymphon spinosissimum Norman, 1894
- RBCM 975-00136-002 (3 males)

A.2.5 Family Phoxichilidiidae Sars, 1891

Anoplodactylus viridintestinalis (Cole, 1904)
- RBCM 983-00022-004 (1 female)

Phoxichilidium femoratum (Rathke, 1799)
- RBCM 976-01081-027 (1 female)
- RBCM 977-00155-006 (1 male)
- RBCM 982-00243-004 (1 male)

Phoxichilidium parvum Hilton, 1939
- RBCM 973-00156-033 (1 male)
- RBCM 973-00157-033 (3 males, 1 female)

Phoxichilidium quadradentatum Hilton, 1942f
- RBCM 977-00145-005 (1 male, 1 female)
- RBCM 984-00102-008 (1 female)
- RBCM 001-00060-001 (1 male, 1 female)

A.2.6 Family Pycnogonidae Wilson, 1878

Pycnogonum rickettsi Schmitt, 1934
- RBCM 975-00001-014 (1 male)

Pycnogonum stearnsi Ives, 1892
- RBCM 976-00028-003 (1 female)

A.3 Catalogue of Potential British Columbia Pycnogonida

- based on published and unpublished records of species collected from Alaska to California
- asterisk indicates species reported from BC waters
- indented species represent species synonyms

A.3.1 Family Ammotheidae Dohrn, 1881

Achelia alaskensis (Cole, 1904) *
    Ammothea alaskensis Cole, 1904
    Ammothea nudiuscula Hall, 1913
    Achelia nudiuscula (Hall, 1913)
    Achelia gurjanovii Losina-Losinsky, 1961
    Achelia kamtschatica Losina-Losinsky, 1961
Achelia borealis (Schimkewitsch, 1893)
    Ammothea borealis Schimkewitsch, 1893
    Ammothea borealis var. *japonica* Losina-Losinsky, 1933
    Ammothea elongata Hilton, 1942g
Achelia brevirostris Losina-Losinsky, 1961
Achelia chelata (Hilton, 1939) *
    Ammothea chelata Hilton, 1939
    Ammothea euchelata Hedgpeth, 1940
Achelia cuneatis Child, 1999
Achelia discoidea (Exline, 1936) *
Ammothea discoidea Exline, 1936
Achelia echinata Hodge, 1864 *
    Achelia fibulifera Dohrn, 1881
Achelia euryfrontalis Turpaeva, 2000
Achelia gracilipes (Cole, 1904) *
    Ammothea gracilipes Cole, 1904
Achelia latifrons (Cole, 1904) *
    Ammothea latifrons Cole, 1904

- *Achelia longicaudata* Stimpson, 1864 *
  - *Ammothea longicaudata* Stimpson, 1864
  - ** Nomen dubium - specimen lost **
- *Achelia megova* (Hilton, 1942g)
  - Ammothea megova Hilton, 1942g
- *Achelia ovosetosa* (Hilton, 1942g)
  - *Ammothea ogosetosa* Hilton, 1942g
- *Achelia pribilofensis* (Cole, 1904)
  - *Ammothea pribilofensis* Cole, 1904
- *Achelia rostrata* Turpaeva, 2000
- *Achelia simplissima* (Hilton, 1939)
  - *Ammothea simplissima* Hilton, 1939
  - *Ammothea simplisicma* Hilton, 1942g
- *Achelia spinoseta* (Hilton, 1939)
  - Ammothea spinoseta Hilton, 1939
- *Achelia superba* (Loman, 1911)
  - *Ammothea superba* Loman, 1911
- *Ammothea hilgendorfi* (Böhm, 1879) *
  - *Corniger hilgendorfi* Böhm, 1879
  - *Lecythorhynchus marginatus* Cole, 1904
  - *Ammothea marginatus* (Cole, 1904)
  - *Lecythorhynchus ovatus* Hilton, 1942d
- *Ammothea dorsiplicata* (Hilton, 1942g)
  - *Leionymphon dorsiplicatum* Hilton, 1942g
- *Ammothea verenae* (Child, 1987)
  - *Scipiolus thermophilus* Turpaeva, 1988
- *Ammothella biunguiculata* (Dohrn, 1881)
  - *Ammothella biunguiculata* var. *californica* Hall, 1912
  - *Ammothella biunguiculata* var. *fusca* Hilton, 1942c
- *Ammothella heterosetosa* Hilton, 1942b
- *Ammothella menziesi* Hedgpeth, 1951
- *Ammothella pacifica* Hilton, 1942d
- *Ammothella setosa* Hilton, 1942d
  - *Ammothella killix* Dojiri, Cadien & Phillips, 1991
- *Ammothella spinifera* Cole, 1904
- *Ammothella tuberculata* Cole, 1904 *
- *Ascorhynchus agassizii* Schimkewitsch, 1893
- *Ascorhynchus japonicum* Ives, 1891 *
  - *Ascorhynchus* japonicus Ives, 1891
- *Ascorhynchus laterospinus* Hilton, 1942g
- *Eurycyde arctica* Child, 1995a
- *Eurycyde depressa* Child, 1995a
- *Eurycyde longisetosa* Hilton, 1942b
- *Eurycyde muricata* Child, 1995a
- *Eurycyde spinosa* Hilton, 1916
- *Nymphopsis duodorsospinosa* Hilton, 1942b

- *Nymphopsis spinosissima* (Hall, 1912) *
    - *Ammothella spinosissima* Hall, 1912
    - *Nymphopsis spinosissimus* Hedgpeth, 1939
- *Prototrygaeus jordanae* Child, 1990b
- *Sericosura cyrtoma* Child and Segonzac, 1996
- *Sericosura dissita* Child, 2000
- Sericosura *venticola* Child, 1987
- *Tanystylum bealensis* Gillespie and Bain, 2004 *
- *Tanystylum californicum* Hilton, 1939
- *Tanystylum duospinum* Hilton, 1939
    - *Tanystylum oculospinosum* Hilton, 1942e
    - *Tanystylum tubirostre* Stock, 1954a
    - *Tanystylum tubirostrum* Stock, 1975
    - *Tanystylum mexicanum* Child, 1979
- *Tanystylum grossifemora* (Hilton, 1942g) *
    - *Ammothea grossifemora* Hilton, 1942g
    - *Tanystylum anthomasthi* Hedgpeth, 1949
    - *Tanystylum anthomasti* Hedgpeth, 1963
    - *Tanystylum grossifemorum* Child, 1995a
- *Tanystylum intermedium* Cole, 1904
    - *Tanystylum panamum* Hilton, 1942e
    - *Tanystylum intermedioides* Hedgpeth, 1961
- *Tanystylum nudum* Hilton, 1939
    - ** Nomen dubium - specimen lost **
- *Tanystylum occidentalis* (Cole, 1904) *
    - *Clotenia occidentalis* Cole, 1904
- *Tanystylum orbiculare* Wilson 1878

A.3.2 Family Callipallenidae Hilton, 1942c

- *Anoropallene palpida* (Hilton, 1942c) *
    - *Palene palpida* Hilton, 1939
    - *Oropallene palpida* Hilton, 1942c
    - *Oropallene heterodenta* Hilton, 1942c
    - *Anoropallene heterodenta* (Hilton, 1942c)
    - *Anoropallene crenispina* Stock, 1956a
- *Callipallene californiensis* (Hall, 1913)
    - *Pallene californiensis* Hall, 1913
    - *Palene californiensis* Hilton, 1915
    - *Callipallene solicitatus* Child, 1979
- *Callipallene pacfica* (Hedgpeth, 1939)
    - *Pallene pacifica* Hedgpeth, 1939
- *Decachela discata* Hilton, 1939
- *Oropallene ovigerosetosus* Hilton, 1942d
    - *Callipallene ovigerosetosa* Hilton, 1942c

- *Pallenopsis* (*Bathypallenopsis*) *californica* Schimkewitsch, 1893
- *Pallenopsis* (*Bathypallenopsis*) *comosa* Stock, 1975
- *Pallenopsis* (*Bathypallenopsis*) *longiseta* Turpaeva, 1957
  - *Pallenopsis mollissima* Schimkewitsch, 1893
- *Pallenopsis* (*Bathypallenopsis*) *oculotuberculosis* Hilton, 1942c
- *Pallenopsis* (*Bathypallenopsis*) *pacifica* Hilton, 1942c
  - *Pallenopsis stschapovae* Turpaeva, 1957
- *Pallenopsis profundis* Hilton, 1942c
- *Pallenopsis* (*Bathypallenopsis*) *stylirostris* Hedgpeth, 1949
  - *Pallenopsis stylirostre* Hedgpeth, 1949
- *Pigrogromitus timsanus* Calman, 1927
  - *Clotenopsa prima* Hilton, 1942d
- *Pigrogromitus robustus* Hilton, 1942c
- *Pseudopallene circularis* (Goodsir, 1842) *
  - *Pallene circularis* Goodsir, 1842
  - *Cordylochele microspines* Hilton, 1942c
  - *Cordylochele setospines* Hilton, 1942c
  - *Pseudopallene setosa* Hilton, 1942c
  - *Pseudopallene spinosa* Hilton, 1942c

A.3.3 Family Colossendeidae Hoek, 1881

- *Colossendeis angusta* Sars, 1877 *
  - *Colossendeis gracilis* Hoek, 1881
- *Colossendeis bicenata* Schimkewitsch, 1893
- *Colossendeis colossea* Wilson, 1881 *
- *Colossendeis spinifera* Hilton, 1943a
- *Colossendeis cucurbita* Cole, 1909
- *Colossendeis dalli* Child, 1995a
- *Colossendeis japonica* Hoek, 1881 *
- *Colossendeis leptorhynchus* Hoek, 1881
- *Colossendeis macerrima* Wilson, 1881 *
- *Colossendeis microsetosa* Hilton, 1943a
  - *Colossendeis orientalis* Losina-Losinsky & Turpaeva, 1958
- *Colossendeis peloria* Child, 1994 *
- *Colossendeis specula* Child, 1994
- *Colossendeis tenera* Hilton, 1943c *
- *Hedgpethia articulata* (Loman, 1908)
  - *Colossendeis articulata* Loman, 1908
- *Hedgpethia californica* (Hedgpeth, 1939) *
  - *Colossendeis californica* Hedgpeth, 1939
  - *Colossendeis bicornis* Losina-Losinsky & Turpaeva, 1958
- *Hedgpethia chitinosa* (Hilton, 1943a) *
  - *Colossendeis chitinosa* Hilton, 1943a
  - *Rhopalorhynchus chitinosum* Stock, 1970
  - *Hedgpethia californica chitinosa* Turpaeva, 1973

- *Hedgpethia dofleini* (Loman, 1911) *
    - *Colossendeis dofleini* Loman, 1911
    - *Rhopalorhynchus dofleini* Stock, 1958
- *Hedgpethia nasica* Child, 1994

A.3.4 Family Endeidae Norman, 1908

- *Endeis nodosa* Hilton, 1942d

A.3.5 Family Nymphonidae Wilson, 1878

- *Heteronymphon bioculatum* Turpaeva, 1956
- *Heteronymphon horikoshii* Nakamura, 1985
- *Nymphon aculeatum* Child, 1994
- *Nymphon basispinosum* Hilton, 1942a
- *Nymphon bergi* Losina-Losinsky, 1961
- *Nymphon brevitarse* Krøyer, 1838
    - *Nymphon microcollis* Hilton, 1942a
- *Nymphon duospinum* (Hilton, 1942a)
    - *Chaetonymphon duospinum* Hilton, 1942a
    - *Chaetonymphon quadrispinum* Hilton, 1942a
- *Nymphon elongatum* Hilton, 1942a
- *Nymphon gracilipes* Miers, 1875
- *Nymphon grossipes* (Fabricius, 1780) *
    - *Nymphon turritum* Exline, 1936
    - *Nymphon heterodenticulatum* Hedgpeth, 1941
    - *Nymphon nigroanathum* Hilton 1942a
    - *Nymphon oculospinum* Hilton, 1942a
- *Nymphon hitsutum* Child, 1995a
- *Nymphon longitarse* Krøyer, 1844 *
- *Nymphon microsetosum* Hilton, 1942a
- *Nymphon mixtum* Krøyer, 1844
- *Nymphon molum* Hilton, 1942a
- *Nymphon profundum* Hilton, 1942a
    - *Nymphon noctum* Hilton, 1942a
- *Nymphon pixellae* Scott, 1913 *
    - *Nymphon solitarium* Exline, 1936
    - *Nymphon variatum* Hilton, 1942a
- *Nymphon procerum* Hoek, 1881
- *Nymphon rubrum* Hodge, 1865 *
- *Nymphon stipulum* Child, 1990a

A.3.6 Family Phoxichilidiidae Sars, 1891

Anoplodactylus batangensis (Helfer ????)
    Anoplodactylus intermedius Hilton, 1942d
    Anoplodactylus batagensis Stock, 1968
Anoplodactylus californicus Hall, 1912
    Nymphon dubium Nicolet, 1849
    Anoplodactylus portus Calman, 1927
    Anoplodactylus robustus Hilton, 1939
    Anoplodactylus carvalhoi Marcus, 1940
Anoplodactylus californiensis Hedgpeth, 1941
    Anoplodactylus projectus Hilton, 1942d
Anoplodactylus compactus (Hilton, 1939)
    Phoxichildium compactum Hilton, 1939
    Halosoma compactum Hilton, 1942f
Anoplodactylus erectus Cole, 1904 *
Anoplodactylus nodosus Hilton, 1939
Anoplodactylus oculospinus Hilton, 1942f
Anoplodactylus pacificus Hilton, 1942f
Anoplodactylus petiolatus Stephensen, 1935
Anoplodactylus pycnosoma (Helfer 1938)
    Peritrachia pycnosoma Helfer, 1938
    Halosoma pycnosoma Marcus, 1940
    Phoxichildium truncatum Hilton, 1942d
    Pallenopsis truncatum Hilton, 1942d
Anoplodactylus typhlops Sars, 1888
Anoplodactylus viridintestinalis (Cole, 1904) *
    Halosoma viridintestinalis Cole, 1904
    Phoxichilus compactus Hilton, 1939
    Endeis compacta Hilton, 1943b
Phoxichilidium femoratum (Rathke, 1799) *
    Nymphon femoratum Rathke, 1799
    Orithyia coccinea Johnston, 1837
    Phoxichilidium globosum Goodsir, 1842
    Phoxichilidium maxillare Wilson, 1878
    Phoxichildium minor Wilson, 1878
Phoxichilidium micropalpidum Hilton, 1942f
Phoxichilidium parvum Hilton, 1939 *
    Phoxichilidium hokkaidoense Utinomi, 1954
Phoxichilidium quadradentatum Hilton, 1942f *
    Phoxichildium quadridentatum Hedgpeth, 1954
Phoxichilidium ungellatum Hedgpeth, 1949
Pycnosoma strongylocentroti Losina-Losinsky 1933
    Pigrogromitus robustus Hilton, 1942c

A.3.7 Family Pycnogonidae Wilson 1878

Pycnogonum rickettsi Schmitt, 1934 *
    Pycnogonum rickettii Schmitt, 1934
Pycnogonum stearnsi Ives, 1892 *
Pycnogonum stylidium Child, 1995a
Pycnogonum tenue (Slater, 1879)
    Pycnogonum littorale var. *tenue* Slater, 1879
Pycnogonum ungellatum Loman, 1911

A.3.8 Family Rhynchothoracidae Thompson, 1908

Rhynchothorax philopsammum Hedgpeth, 1951

A.4 Key to the Pycnogonid Families (taken from Child, 1998b)

- designed for use with mature (adult) specimens, either sex

1. Chelifores and palps present; ovigers in both sexes → 2

Chelifores or palps, or both, lacking or greatly reduced; ovigers in both sexes, in male only or lacking → 4

2(1). Chelifores or chelae, or both, vestigial, some achelate; palps 4- or 10-segmented, ovigers 9- or 10-segmented, with or without terminal claw, with denticulate or simple spines → AMMOTHEIDAE (p. 103)

Chelifores and chelae well developed, functional → 3

3(2). Palps with 5-segments; ovigers with 10, with strong strigilis, terminal claw and denticulate spines; propodus with or without auxiliary claws → NYMPHONIDAE (p. 115)

Palps lacking, reduced to buds, or 4-segmented; oviger with 9- or 10-segments, strigilis with simple or denticulate spines, with or without terminal claw; propodus with or without auxiliary claws → CALLIPALLENIDAE (p. 108)

4(1). Chelifores present; palps absent; ovigers 5- or 6-segmented, in males only, without strigilis or terminal claw; auxiliary claws tiny or lacking → PHOXICHILIDIIDAE (p. 117)

Chelifores lacking; palps present or lacking; ovigers in both sexes or males only, or lacking altogether, with or without terminal claw → 5

5(4). Size giant, appendages extremely long; palps and ovigers 10-segmented, with terminal claw and rows of denticulate spines; auxiliary claws lacking → COLOSSENDEIDAE (p. 113)

Size much smaller, appendages shorter; palps present or lacking --> 6

6(5). Proboscis long, pipette-like, with rings over most of its length; palps usually 6-segmented; ovigers 1- to 6-segmented, not functional, without claw, lacking in some females → AUSTRODECIDAE (p. 108)

Proboscis more typical size and shape; palps usually of 4-segments or lacking; ovigers of 7- to 9-segments, in both sexes, in males only, or entirely lacking in some → 7

7(6). Size very tiny; lateral processes crowded; lips of proboscis oral surface vertical; palps 4-segmented; 8-segmented ovigers, usually in both sexes → RHYNCHOTHORACIDAE (p. 119)

Size little larger; lateral processes crowded or separated; oral surface round; palps lacking entirely; ovigers with 7- or 9-segments in males only or lacking → 8

8(7). Habitus slender (*Anoplodactylus*-like); ovigers 7-segmented, in males only, strigilis weak, without claw, with setae only; multiple cement gland pores → ENDEIDAE (p. 115)

Habitus stout, with short robust legs; ovigers 9-segmented, lacking in males of few species, with terminal claw; usually with single lateral cement gland pore on first coxae → PYCNOGONIDAE (p. 118)

A.5 Family AMMOTHEIDAE Dohrn, 1881

A.5.1 Diagnostic Characteristics

- Chelifores: present (chelate or achelate), absent
- Palps: 4- to 10-segmented; both sexes
- Ovigers: 9- or 10-segmented; both sexes

A.5.2 Genera and number of species: 31 genera, 373 species
- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Ascorhynchus* - 71 sp.
Achelia* - 69 sp.
Tanystylum* - 44 sp.
Ammothella* - 41 sp.
Ammothea* - 31 sp.
*Cilunculus** - 26 sp.
*Eurycyde** - 18 sp.
Nymphopsis* - 13 sp.

Achieliana - 1 sp.
Austroraptus - 5 sp.
Bathyzetes - 3 sp.
Biammothea - 1 sp.
Boehmia - 4 sp.
Calypsopycnon - 1 sp.
Chonothea - 2 sp.
Dromedopycnon - 1 sp.
Elassorhis - 1 sp.
Ephryogymna - 1 sp.
Hedgpethius - 3 sp.
Hemichela - 2 sp.
Heterofragilia - 5 sp.
Megarhetus - 1 sp.
Nymphonella - 2 sp.
Oorhynchus - 1 sp.
Paranymphon - 3 sp.
Proboehmia - 1 sp.
Prototrygaeus - 3 sp.
Pycnofragilia - 1 sp.
Scipiolus - 3 sp.
Sericosura - 6 sp.
Trygaeus - 1 sp.

A.5.3 Key to the Ammotheidae (taken from Child, 1979)
- designed for use with mature (adult) specimens, either sex

1. Body circular or discoidal, lateral processes touching or only narrowly separated distally; scape 1-segmented; palps 4- to 8-segmented → 2

Body more slender, lateral processes separated by half their length or widely separated; scape with 1 or 2 segments; palps 9- or 10-segmented → 3

2(1). Palp 8-segmented; chela present but vestigial; proboscis pyriform; 1st and 2nd coxae with tall dorsal tubercle → *Achelia*

Palp with 4 to 7 segments; chela usually absent, scape reduced to short knob; proboscis usually a tapering cylinder or tubular; 1st coxae only with low rounded tubercles → *Tanystylum*

3(1). Proboscis of 2 segments, a proximal cylinder articulated with pyriform proboscis; most appendages with large pointed spines; abdomen large, spinose; chela vestigial; palps and ovigers 10-segmented; without auxiliary claw → *Eurycyde*

Proboscis of usual single segment, pyriform, ovoid, or cylindrical, with constrictions → 4

4(3). Proboscis ovoid or modified cylindrical; palps of 9 segments → 5

Proboscis pyriform; palps 9- or 10-segmented → 6

5(4). Proboscis ovoid, egg-shaped; trunk with 2 tall median tubercles; legs with tall setose tubercles; chelifores with 3 segments; abdomen long, bent posteriorly → *Nymphopsis*

Proboscis cylindrical, ends constricted; tall tubercles lacking; chelifores blunt, with 2 segments; ocular tubercle a low cone; abdomen short, blunt → *Ammothea*

6(4). Palps 10-segmented, first 2 segments tiny; chelifores 2-segmented, small, chela vestigial; propodus without heel or large spines, without auxiliary claws; dorsal trunk segments with large flaring posterior rims → *Ascorhynchus*

Palps 9-segmented; chelifores 2- or 3-segmented, large, propodus with large heel spines and auxiliary claws; trunk segments without flaring rims → 7

7(6). With hooded anterior cephalic segment dorsal to chelifores → *Cilunculus*

Without hooded anterior cephalic segment → *Ammothella*

A.5.4 Notes on Ranges of Genera

Ascorhynchus
- worldwide; deep-sea
- Sources: Hedgpeth (1948); Fry & Hedgpeth (1969); Stock (1978); Bamber & Thurston (1995); Child (1998b)

Achelia
- found worldwide, but almost half their number are concentrated around the perimeter of the Pacific
- littoral to shallow water
- Sources: Hilton (1942b); Hedgpeth (1948); Fry & Hedgpeth (1969); Bamber & Thurston (1995); Child (1995a); Child (1998a,b)

Tanystylum
- most species of this genus are known from temperate shallow localities in the northern hemisphere.
- littoral to shallow water
- Sources: Hilton (1942b); Hedgpeth (1948); Stock (1956a); Clark (1977); Child (1998b)

Ammothella
- worldwide, shallow water
- Sources: Hedgpeth (1948); Stock (1978); Bamber & Thurston (1995)

Ammothea

- predominantly Antarctic and subantarctic; of the 31 species, 19 are from southern polar (Antarctic and subantarctic) seas.
- only five species are found in the northern hemisphere, and three of these are known only from Japan.
- Sources: Stock (1955); Fry & Hedgpeth (1969); Dojiri *et al.* (1991); Child (1998a,b)

Cilunculus

- Antarctic, Australia, Japan; deep-sea
- Sources: Hedgpeth (1949); Fry & Hedgpeth (1969); Stock (1978); Dojiri *et al.* (1991); Bamber & Thurston (1995); Child (1998b)

Eurycyde

- Caribbean, Antarctic, Pacific, N. Atlantic, Arctic
- Sources: Hilton (1942b); Hedgpeth (1948); Stock (1954b); Child (1988)

Nymphopsis

- North Pacific, NW Atlantic, Southern Africa, W Indian Ocean, Caribbean, Australia
- littoral to ??
- Sources: Hilton (1942b); Hedgpeth (1948); McCloskey (1967)

Austroraptus

- Antarctic and subantarctic, shallow-water
- Sources: Stock (1956a); Fry & Hedgpeth (1969); Child (1998a)

Bathyzetes

- W Indian Ocean, Central Indo-West Pacific
- Sources: Stock (1954b)

Calypsopycnon

- Unknown; museum species with no locality data
- Sources: Hedgpeth (1948)

Chonothea

- Japan, shallow water
- Sources: Nakamura & Child (1983, 1991)

Dromedopycnon

- SW Atlantic, shelf to slope
- Sources: Child (1982a)

Elassorhis

- NW South Atlantic, slope
- Sources: Child (1982a)

Ephryogymna
- deep water (525 fms) formed, dredged off Martinique
- Sources: Hedgpeth (1943, 1948)

Hedgpethius
- Caribbean, intertidal and subtidal
- Sources: Child (1974); Child (1982b)

Hemichela
- Indonesia, Kei Islands, 35 m. Bottom: sandy mud with shells.
- Sources: Stock (1955);

Heterofragilia
- W Pacific, Caribbean; shallow water to abyssal
- Sources: Hedgpeth (1943, 1948)

Megarhetus
- North Atlantic; deep-sea
- Sources: Child (1982a)

Nymphonella
- Japan; parasite inside of clams
- Sources: Hedgpeth (1947)

Oorhynchus
- collected off Auckland, New Zealand in 1280m
- Sources: Child (1998b)

Paranymphon
- NW Atlantic, Mediterranean, NW Pacific; deep-sea
- Sources: Caullery (1896); Hedgpeth (1948); Bamber & Thurston (1995)

Prototrygaeus
- Caribbean; Eastern Pacific; shallow-water
- Sources: Stock (1974); Child (1992)

Scipiolus
- Indonesia, Kei Islands, 90m
- New Zealand, Norfolk Ridge, 450m
- Sources: Doiji *et al.* (1991); Child (1998b)

Sericosura
- Pacific Ocean; Antarctic
- bathyal, often near hydrothermal vents
- Sources: Fry & Hedgpeth (1969)

A.6 Family AUSTRODECIDAE Stock, 1954b

A.6.1 Diagnostic Characteristics

- Chelifores: absent
- Palps: 5- to 8-segmented; both sexes
- Ovigers: 1- to 10-segmented; both sexes

A.6.2 Genera and number of species: 2 genera, 55 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Austrodecus *- 39 sp *Pantopipetta* *- 16 sp.

6.3 Key to the Austrodecidae

- based on Stock (1955); Hedgpeth & McCain (1971); Child (1998b)
- designed for use with mature (adult) specimens, either sex

1. Ocular tubercle strong, reaching far over the implantation of the proboscis; palps 5- or 6-segmented; ovigers strongly reduced in both species, 4- to 7-segmented → *Austrodecus*

Ocular tubercle erect; palps 7- or 8-segmented; ovigers 10-segmented (4-5 segmented in one species) → *Pantopipetta*

A.6.4 Notes on Ranges of Genera

Austrodecus

- Ranging from Antarctic, New Zealand, New Guinea, Philippines, Japan
- Shallow to deep water
- Leg Span: 3-8mm
- Sources: Stock (1955); Child (1998a)

Pantopipetta

- Southern Hemisphere
- Deep water
- Leg Span: 15-30mm
- Sources: Stock (1963); Hedgpeth & McCain (1971)

A.7 Family CALLIPALLENIDAE Hilton, 1942c

A.7.1 Diagnostic Characteristics

- Chelifores: present, chelate
- Palps: 0-, 1-, 4-segmented; males only
- Ovigers: 9- or 10-segmented; both sexes

A.7.2 Genera and number of species: 25 genera, 215 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Pallenopsis* - 80 sp.
Callipallene* - 30 sp.
*Parapallene** - 19 sp.
Pseudopallene* - 12 sp.
*Propallene** - 11 sp.
*Austropallene** - 10 sp.

Anoropallene* - 3 sp.
Bradypallene - 1 sp.
Cheilopallene - 7 sp.
Clavigeropallene - 1 sp.
Decachela - 2 sp.
Hannonia - 4 sp.
Mimipallene - 1 sp.
Neopallene - 3 sp.
*Oropallene** - 5 sp.
Pallenella - 1 sp.
Pallenoides - 6 sp.
*Pigrogromitus** - 1 sp.
Pycnopallene - 1 sp.
Pycnothea - 2 sp.
Queubus - 1 sp.
Safropallene - 1 sp.
Seguapallene - 6 sp.
Spasmopallene - 3 sp.
Stylopallene - 4 sp.

A.7.3 Key to the Callipallenidae (taken from Child, 1982a)

- designed for use with mature (adult) specimens, either sex

1. With segmented palps or palp tubercles → 2

Without trace of palps → 9

2(1). Ovigers with compound (denticulate) spines) → 3

Ovigers with simple or spatulate spines → 6

3(2). Ovigers with terminal claw; auxiliary claws present →4

Ovigers without terminal claw; auxiliary claws absent (except in some *Anoropallene* species) → 5

4(3). Palps 4-segmented, in males only → *Oropallene*

Palps 1-segmented, in males only → *Neopallene*

5(3). Palps 4-segmented, in males only → *Anoropallene*

Palps 2-segmented, in males only → *Propallene*

6(2). Ovigers without terminal claw; auxiliary claws present → 7

Ovigers with terminal claw; auxiliary claws absent → 8

7(6). Oviger terminal segment with setae only; chelifores of 2 or 3 segments, chelae well developed → *Pallenopsis*

Oviger terminal segment with a spatulate spine; chelifores tiny, 1-segmented, without chelae → *Mimipallene*

8(6). Trunk and legs very spinose; lateral processes separated by less than their own diameter; tarsus much shorter than propodus → *Hannonia*

Trunk and legs glabrous; lateral processes widely separated; tarsus equal to or longer than propodus; *Pycnogonum*-like, but with chelifores → *Pycnopallene*

9(1). Without trace of chelifores; *Pycnogonum*-like → ***Queubus***

Chelifores well developed → 10

10(9). Scape 2-segmented; chela fingers smooth; oviger with terminal claw; auxiliary claws absent → *Pigrogromitus*

Scape 1-segmented → 11

11(10). Oviger with terminal claw → 12

Oviger without terminal claw →17

12(11). Chela fingers with 1 or more median tubercles or nodes giving denticulate appearance; proboscis with 3 prominent petal-shaped lips → *Cheilopallene*

Chela fingers smooth or with regular teeth → 13

13(12). Chela fingers with regular serrate teeth → *Seguapallene*

Chela fingers smooth, without teeth → 14

14(13). Long or intermediate neck with "collar"; lips usually project from truncated proboscis; Auxiliary claws absent (but present in *P. bermudensis* & *P. challengeri*) → *Parapallene*

Neck very short; auxiliary claws absent → 15

15(14). Proboscis conical distally → *Pseudopallene*

Proboscis reduced to tube distally → 16

16(15). Chelae and proboscis carried anteriorly; distal proboscis a tube with tiny triangular lips → *Stylopallene*

Proboscis and chelifores carried under trunk; chela carried anaxially → *Spasmopallene*

17(11). Chelae with serrate or denticulate fingers → 18

Chelae fingers smooth, without teeth → 19

18(17). Auxiliary claws present, usually long → *Callipallene*

Auxiliary claws absent or very short → *Pallenoides*

19(17). Auxiliary claws present → *Pycnothea*

Auxiliary claws absent → 20

20(19). Propodus with 1 large basal spine giving chelate appearance with claw; *Achelia*-like → *Decachela*

Propodus without large spine; proboscis styliform; tubercles on chelifores and lateral processes → *Austropallene*

A.7.4 Notes on Ranges of Genera

Pallenopsis

- worldwide, but are predominantly more common in the southern hemisphere with an abundance in the subantarctic and Antarctic forms
- range from subtidal to 2000+m; very few have been collected in the littoral
- subgenus *Bathypallenopsis* has some pelagic species, living on planktonic medusae
- Sources: Wilson (1881); Hedgpeth (1948); Stock (1955); Child (1994, 1998a,b)

Callipallene

- worldwide in relatively shallow waters, with a few deep-sea species
- Sources: Hedgpeth (1948); Stock (1955); Child (1979, 1998b)

Parapallene

- majority of species are concentrated around the periphery of Australia and the east and south African coast, but two are also found in the Atlantic and several in the western Pacific islands.
- most are at intertidal depths (<100m), with several found in deeper waters
- Sources: Stock (1955); Child (1998b)

Pseudopallene
- Maine, USA; 12 fathoms
- Boreal-Arctic; Australia; South Africa; Malaysia
- Sources: Wilson (1878); Hedgpeth (1948); Stock (1955)

Propallene
- Japan
- Sources: Schimkewitsch (1909); Stock (1955)

Austropallene
- subantarctic, Antarctic & New Zealand
- shallow to deep water
- Sources: Hodgson (1915); Child (1995d, 1998a,b)

Anoropallene
- NW & Central east Pacific Ocean, Australia
- Sources: Stock (1956b); Clark (1963);

Chelopallene
- only known from the Indo-West Pacific, Caribbean & Antarctica
- Sources: Stock (1954b); Child (1998b)

Hannonia
- NE Atlantic, W Indian Ocean
- Sources: Munilla (1993)

Mimipallene
- SW Atlantic; abyssal
- Sources: Child (1982a)

Neopallene
- Mediterranean, Azores & New Zealand
- shallow to intermediate depths
- Sources: Stock (1955); Child (1998b)

Oropallene
- majority of species found in the SW Pacific and Indian Oceans; also New Zealand, Australia Kerguelen Island and Alaska
- shallow to deep-sea
- Sources: Wood-Mason (1873); Stock (1955); Clark (1963); Child (1998b)

Pallenella
- Australia
- Sources: Schimkewitsch (1909)

Pallenoides
- SW Atlantic, S. Africa, W. Indian Ocean, Caribbean, SE Australia
- shallow to deep (shelf)
- Sources: Stock (1951, 1954b)

Pigrogromitus
- Florida, intertidal; Suez Canal
- Sources: Hedgpeth (1948)

Pycnothea
- South Pacific Ocean
- Sources: Loman (1920)

Queubus
- Southern Africa; littoral to deep-sea
- Sources: Barnard (1946)

Seguapallene
- Australia, Southern Ocean (Kerguelen Island), Central West Pacific, W. Atlantic
- shallow to intermediate depths
- Sources: Pushkin (1975); Child (1983)

Stylopallene
- Australia, shallow water
- Sources: Clark (1963)

A.8 Family COLOSSENDEIDAE Hoek, 1881

8.1 Diagnostic Characteristics

- Chelifores: absent
- Palps: 10-segmented; both sexes
- Ovigers: 10-segmented; both sexes

A.8.2 Genera and number of species: 6 genera, 81 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Colossendeis* - 54 sp
Hedgpethia* - 12 sp.
*Rhopalorhynchus** - 10 sp.
*Decolopoda** - 10 sp.
*Dodecolopoda** - 2 sp.
*Pentacolossendeis** - 1 sp.

A.8.3 Key to the Colossendeidae

- based on Hedgpeth (1948); Fry & Hedgepeth (1969); Child (1998a,b)
- designed for use with mature (adult) specimens, either sex

1. 4 pairs of walking legs → 2

   5 or 6 pairs of walking legs → 4

2(1). Intersegmental sutures present on the trunk → 3

   No intersegmental sutures on the trunk → *Colossendeis*

3(2). Lateral processes separated by less than their diameters from each other → *Hedgpethia*

   Lateral processes separated by much more than their diameters from each other → *Rhopalorhynchus*

4(1). 5 pairs of walking legs → 5

   6 pairs of walking legs → *Dodecolopoda*

5(4). Chelifores present; intersegmental sutures present on the trunk → *Decolopoda*

   Chelifores absent; no intersegmental sutures on the trunk → *Pentacolossendeis*

A.8.4 Notes on Ranges of Genera

Colossendeis
- cosmopolitan deep-sea genus often found in quite shallow localities in Antarctic waters
- Sources: Hedgpeth (1948); Bamber & Thurston (1995); Child (1998a,b); Brusca & Brusca (2002)

Hedgpethia
- very wide depth range among the various species, one is found as shallow as 20m while most range from 600+ m and on is only known from 4100m
- found throughout Pacific, Australia, Artic, NE Atlantic, Southern Africa
- Sources: Child (1998b)

Rhopalorhynchus
- South Pacific, Caribbean, Red Sea
- shallow water
- Sources: Wood-Mason (1873); Stock (1958)

Decolopoda
- Antarctic
- Sources: Child (1998a)

Dodecolopoda
- Antarctic
- Sources: Child (1998a)

Pentacolossendeis
- Florida, 110fms
- Sources: Hedgpeth (1948)

A.9 Family ENDEIDAE Norman, 1908

A.9.1 Diagnostic Characteristics

- Chelifores: absent
- Palps: absent
- Ovigers: 7-segmented; males only

A.9.2 Genera and number of species: 1 genus, 17 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

*Endeis** - 17sp

A.9.3 Notes on Ranges of Genus

Endeis
- found in few numbers around the world oceans and are not very common, except for the genotype which is a resident of floating *Sargassum* weed and is carried vast distances throughout the Atlantic Ocean and Mediterranean Sea.
- Sources: Hedgpeth (1948); Fry & Hedgpeth (1969); Child (1998b)

A.10 Family NYMPHONIDAE Wilson, 1878

A.10.1 Diagnostic Characteristics

- Chelifores: present, chelate
- Palps: 4- or 5-segmented; both sexes
- Ovigers: 10-segmented; both sexes

A.10.2 Genera and number of species: 6 genera, 248 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Nymphon * - 235sp.
Heteronymphon *- 6 sp.
Boreonymphon *- 4 sp.
Neonymphon *- 1 sp.
Pentanymphon *- 1 sp.
Sexanymphon *- 1 sp.

A.10.3 Key to the Nymphonidae

- based on Stock (1954b); Bamber and Thurston (1995); Child (1998b)
- designed for use with mature (adult) specimens, either sex

1. Five or six pairs of legs → 2

Four pairs of legs → 3

2(1). Five pairs of legs → *Pentanymphon* (Antarctic)

Six pairs of legs → *Sexanymphon* (Antarctic)

3(1). Palps 4-segmented, hardly longer than the proboscis → *Neonymphon*

Palps 5-segmented, distinctly longer than the proboscis → 4

4(3). Ocular tubercle at the frontal margin of the cephalic segment. No terminal oviger claw → *Heteronymphon*

Ocular tubercle at the base of the neck. Oviger with terminal claw → 5

5(4). Fingers of chelae with teeth → *Nymphon*

Fingers of chelae without teeth → *Boreonymphon*

A.10.4 Notes on Ranges of Genera

Nymphon

- worldwide in all oceans and all depths, but with increased numbers and more species occurring in Arctic and Antarctic localities followed closely by temperate and tropical habitats
- Sources: Hilton (1942b); Hedgpeth (1948, 1949); Child (1995b;1998a,b)

Heteronymphon

- found for the most part in very deep water, with exceptions
- all are slender and rather small species
- Sources: Child (1998b)

Boreonymphon

- Arctic, subtidal
- Sources: Just (1972)

Neonymphon

- South Pacific Ocean, St. Croix, Virgin Is.
- 500 fathoms
- Sources: Stock (1954b)

Pentanymphon

- Antarctic, extending to New Zealand and Magellanic areas, with a wide depth range of less than 200m to 3227m
- Sources: Hodgson (1904); Hedgpeth (1947); Child (1998a,b)

Sexanymphon

- Antarctic, deep waters
- Sources: Hedgpeth and Fry (1964)

A.11 Family PHOXICHILIDIIDAE Sars, 1891

A.11.1 Diagnostic Characteristics

- Chelifores: present, chelate
- Palps: absent
- Ovigers: 5- or 6-segmented; male only

A.11.2 Genera and number of species: 4 genera, 236 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Anoplodactylus *- 122sp.
Phoxichilidium *- 8 sp.
Pycnosoma *- 4 sp.
Phoxiphilyra - 2 sp.

A.11.3 Key to the Phoxichilidiidae

- based on Hedgpeth (1948); Nakamura & Child (1983); Child (1998b)
- designed for use with mature (adult) specimens, either sex

1. Chelifores longer than proboscis → 2

   Chelifores shorter than proboscis → *Pycnosoma*

2(1). Trunk usually with anterior extension of cephalic segment; propodus usually with Auxiliary claws lacking or with minute Auxiliary claws on the sides of the main claw, shorter than the main claw diameter → *Anoplodactylus*

Trunk without anterior extension of cephalic segment; propodus with Auxiliary claws, occurring on the top of the main claw, longer than the main claw diameter → *Phoxichilidium*

A.11.4 Notes on Ranges of Genera

Anoplodactylus

- worldwide in mostly shallow waters of temperate and tropical seas, but a few species known from Antarctic and subantarctic and several species have been taken from depths greater than 1000m.
- Sources: Wilson (1878); Hedgpeth (1948); Bamber & Thurston (1995); Child (1998b)

Phoxichilidium

- worldwide, subtidal
- Sources: Hedgpeth (1948); Child (1998b)

Pycnosoma

- three of four species known have been collected in association with echinoderms and are apparently commensal or parasitic on these host specimens.
- Indo-West Pacific: boreal-arctic waters of Russian Far East, Philippines
- shallow to moderate depths of 17-591m.
- Sources: Child (1998b)

A.12 Family PYCNOGONIDAE Wilson, 1878

A.12.1 Diagnostic Characteristics

- Chelifores: absent
- Palps: absent
- Ovigers: 9-segmented; males only

A.12.2 Genera and number of species: 2 genera, 63 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Pycnogonum *- 59sp.	*Pentapycnon* *- 1 sp.

A.12.3 Key to the Pycnogonidae

- based on Hedgpeth (1948); Fry & Hedgpeth (1969)
- designed for use with mature (adult) specimens, either sex

1. Four pairs of legs → *Pycnogonum*

   Five pairs of legs → *Pentapycnon*

A.12.4 Notes on Ranges of Genera

Pycnogonum
- world-wide distribution, intertidal to subtidal
- Sources: Hilton (1942b); Hedgpeth (1948); Child (1998a,b)

Pentapycnon
- Antarctic, Caribbean, subtidal
- Sources: Hedgpeth (1947, 1948); Fry & Hedgpeth (1969)

A.13 Family RHYNCHOTHORACIDAE Thompson, 1908

A.13.1 Diagnostic Characteristics

- Chelifores: absent
- Palps: 4-segmented; both sexes
- Ovigers: 8-segmented; both sexes

A.13.2 Genera and number of species: 1 genus, 17 species

- Bold indicates genera that have been collected from the NE Pacific ocean
- Asterisk indicates genera included in key

Rhynchothorax *- 17sp

A.13.3 Notes on Range of Genus

Rhynchothorax
- Isolated populations worldwide: Antarctic, New Zealand, NW Africa, California
- Habitat: interstital shallows, among sand grains, underside of rocks, subtidal
- Depth: 0-2000m
- Sources: Clark (1976); Child (1995c, 1998b)

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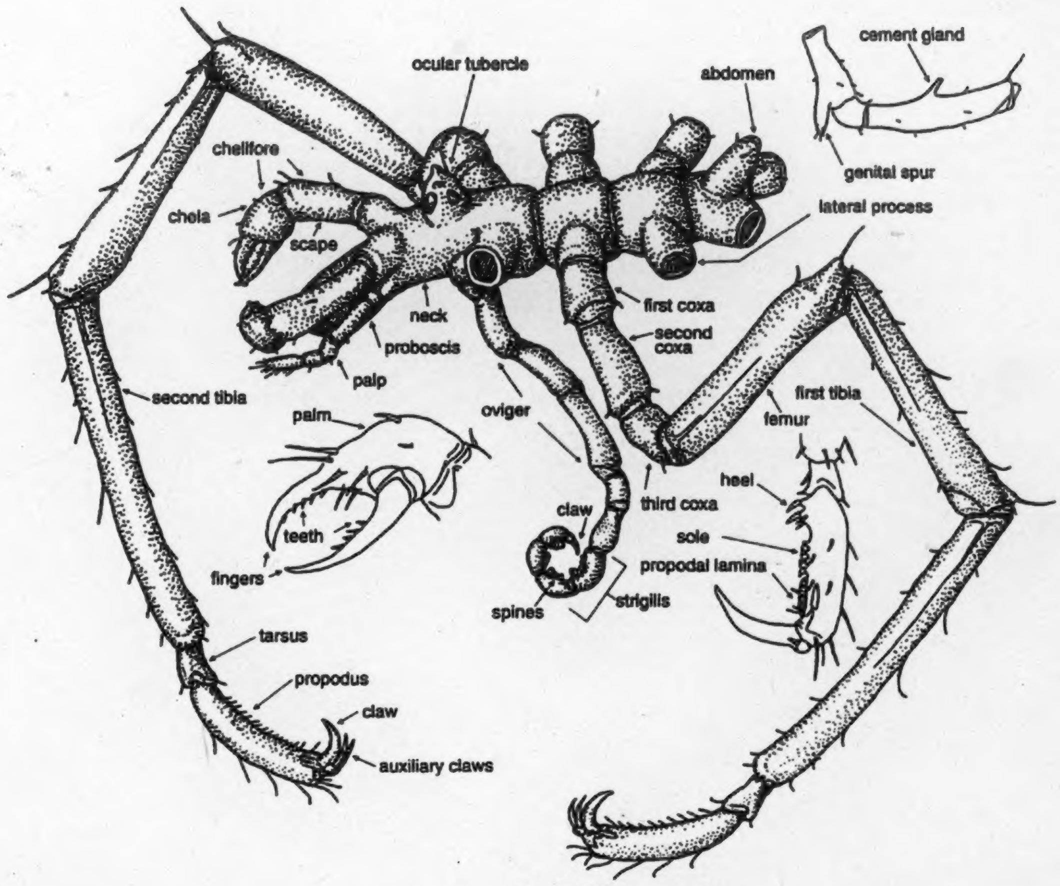
cement gland
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proboscis
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second tibia
first tibia
femur
palm
oviger
heel
teeth
claw
third coxa
sole
propodal lamina
fingers
spines
strigilis
tarsus
propodus
claw
auxiliary claws

Figure A-1. Diagram of the general pycnogonid morphology, showing structures used in the keys. Taken from Child (1998b).

APPENDIX B

Photograph of Joel W. Hedgpeth (right) and JMG, taken at Hedgpeth's home in Santa Rosa, California on May 1, 2001.